THE DETERMINANTS OF NUTRITIONAL RISK IN PAEDIATRIC CANCER

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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Dedication

This thesis is dedicated to both of my grandparents Dr Pedro Revuelta Gómez (†), whose constant curiosity about life and hunger for knowledge remained until his last days, and Mr Francisco Iniesta Atienza (†), whose humility and working etiquette were extremely admirable. Both of you have been my source of inspiration and perseverance.

For his enduring love, support and understanding this thesis is dedicated to my dear husband, Dr Edmund Rhatigan.

Finally I would like to dedicate this thesis to all my family, especially my sister Cristina, my mum Marisa and my dad Dr Pedro Revuelta Rabasa and my two cats.

"It's not that I'm so smart, it's just that I stay with problems longer."

Albert Einstein

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Declaration of Originality

I declare that the work contained within this thesis is original. I have solely been responsible for the data collection, experimental work and subsequent analysis and writing of the thesis herein, with the exception of:

1. The first 26 subjects were recruited and monitored for part of the project by Dr Ilenia Paciarotti
2. The antioxidant capacity and lipid peroxidation analyses were all performed by Foudil Smail in the Biochemistry laboratory from Queen Margaret University
3. The polyunsaturated fatty acid samples were all analysed by Prof Gordon Bell and his team in the Institute of Aquaculture, University of Stirling.
4. The meta-analysis from chapter II, section 2.2 was carried out by Robert Rush.

Raquel Revuelta Iniesta

Abstract

The five-year survival rates of paediatric cancer patients have improved considerably in the last 40 years with the implementation of more intensive and progressive treatments. Consequently attention is shifting to the reduction of treatment-related sequelae during and after the completion of therapy. Malnutrition and vitamin D inadequacy are a major concern as they are thought to increase the risk of short- and long-term complications in this population. Furthermore, emerging evidence has found a protective role of antioxidants and docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) against chronic conditions, including cardiovascular disease and cancer, which are common long-term complications in survivors of paediatric cancer. Therefore, this thesis aimed to investigate the nutritional status (NS), vitamin D, antioxidants and oxidative stress levels, as well as DHA and EPA levels of paediatric cancer patients. Potential factors that may contribute to the development of malnutrition in this population were also investigated.

A prospective cohort-study of SE Scottish children aged <18 years, diagnosed with and treated for cancer between Aug 2010-Jan 2014 was performed. Clinical and nutritional data were collected at defined periods up to 36 months. NS was assessed using anthropometry, bioelectrical impedance analyisis (BIA), plasma micronutrients and dietary intake. DHA, EPA, antioxidant capacity and oxidative stress were measured at baseline and 6 months between April 2013-Jan 2014. Paediatric cancer was stratified by treatment risk (high, medium and low) and by diagnostic criteria. The primary outcome was malnutrition defined as body mass index (BMI) according to UK growth chart centiles; underweight (<2.3rd), overweight (85-95th) and obese (>95th). Vitamin D status was defined by the Endocrine Society Clinical Practice Guidelines (2011); inadequacy (<50nmol/L).

Eighty-two patients [median(IQR) age 3.9(1.9-8.8) years; 56% males)] were recruited. At diagnosis, the prevalence of undernutrition was 13%, overweight 7% and obesity 15%. TSF identified the highest prevalence of undernutrition (15%) and the lowest of obesity (1%). BMI [p<0.001; 95% CI (1.31-3.47)] and FM (BIA) [p<0.05; 95% CI (0.006-0.08)] significantly increased after 3 months of treatment, whilst FFM (BIA) [p<0.05; 95% CI (-0.78-(-0.01)] significantly decreased during the first three months and these patterns remained until the end of the study. High-treatment risk significantly contributed to undernutrition during the first three months of treatment [p=0.04; 95% CI (-16.8-(-0.4)] and solid tumours had the highest prevalence of undernutrition [BMI (17%)]. Vitamin D inadequacy was highly prevalent (64%; 42/65) at both baseline and during treatment (33-50%) and those children who were not supplemented had the lowest vitamin D levels at every stage with median(IQR) levels ranging from 32.0(21.0-46.5)nmol/L to 45.0(28.0-64.5)nmol/L. Paediatric cancer patients had high levels of oxidative stress and low levels of DHA and EPA, especially at baseline. Antioxidant status remained steady at 6 months, however antioxidant capacity increased slightly. Finally, antioxidant levels, antioxidant capacity, oxidative stress and EPA and DHA did not statistically differ between children receiving nutritional support and those who were not.

Arm anthropometry (or BIA) alongside appropriate nutritional supplementation should be implemented in clinical practice due to the high risk of malnutrition (undernutrition and obesity), the changes in body composition (increase in fat mass and reduction in lean mass) and vitamin D inadequacy, as well as the low levels of EPA and DHA seen in this paediatric cancer cohort.

Key words: paediatric, children, cancer, malnutrition, vitamin D, nutritional status, antioxidants, oxidative stress, EPA, DHA.

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List of abbreviations

AA	Arachidonic Acid
AA/DHA	Ratio of AA/DHA
AA/EPA	Ratio of AA/EPA
AGRP	Agouiti-related Protein
ALL	Acute Lymphoblastic Leukaemia
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AML	Acute Myeloid Leukaemia
ANOVA	Analysis of variance
ASA	Ascorbic Acid
ASR	Ascorbyl Radical
BAPEN	British Association for Parenteral and Enteral Nutrition
BDA	British Dietetic Association
BIA	Bioelectrical impedance analysis
BMI	Body Mass Index
BMR	Basal metabolic rate
BT	Brain tumours
C	Centile
CAM	Complementary and Alternative Medicine
CASP	Critical Appraisal Skills Programme
CAT	Catalase
CC	Case-Control
CD25	Alpha-chain of IL-2 receptor on dendritic cells
CHO	Carbohydrates
CI	Confidence Interval

CML	Chronic Myeloid Leukaemia
CNS	Central Nervous System
CRP	C-Reactive Protein
CRT	Cranial Radiation Therapy
CS	Cross-Sectional Study
CSF	Cerebrospinal Fuid
CT	Chemotherapy
cTAC	Corrected Total Antioxidant Capacity
Cu	Copper
CVD	Cardio Vascular Disease
d	day
DBP	Vitamin D biding protein
Dexa	Dexamethasone
DEXA	Dual-energy X-ray absorptiometry
DH	Department of Health
DHA	Docosahexaenoic acid
DIT	Dietary Induced Thermogenesis
DNA	Deoxyribonucleic Acid
DRV	Dietary Reference Value
Dx	Diagnosis
EAR	Estimate Average Requirements
EDI	Estimated Dietary Intake
EFS	Event Free Survival
EI	Energy Intake
EN	Enteral nutrition
ENRICC	Edinburgh Nutritional Risk in Childhood Cancer

EPA	Eicosapentanoic acid
ESPEN	European Society of Enteral and Parenteral Nutrition
ESPGHAN	European Society for Paediatric Gastroenterology and Nutrition
ETF	Enteral Tube Feeding
FAME	Fatty Acid Methyl Esters
FFM	Fat Free Mass
FFM%	Fat Free Mass percentage
FFQ	Food Frequency Questionnaire
FM	Fat Mass
FM%	Fat Mass percentage
FR	Free Radicals
FRAP	Ferric Reducing Ability of Plasma
g/L	Grams per Litre
GGT	G-glutamyl Transferase
GPx	Glutathione Peroxidase
H	Hydrogen
h	height
HD	Hodgkin’s Disease
HDL	High-density Lipoprotein
HLH	Haemophagocytic Lymphohistiocytosis
HFW	Height for Age
HM	Haematological Malignancies
HSCT	Haematopoietic Stem Cell Transplantation
Ht	height
I	Impedance
ICC	Interclass Correlation Coefficient

ICCC-3	International Classification of Cancer 3rd edition
ICW	Intra cellular water
IFN	Interferon
IL-6	Interleukin-6
IQR	Inter quartile range
Kcal	Kilocalories
Kg	Kilogram
KHz	Kilohertz
LCH	Langerhans Cell Histiocytosis
LC n-3 PUFA	Long-chain Omega-3 Polyunsaturated Fatty Acids
LC n-6 PUFA	Long-chain omega-6 Polyunsaturated Fatty Acids
LDL	Low-density Lipoprotein
M	Method
MDA	Malondialdehyde
mg/L	Miligrams per Litre
mL	millilitres
mmol/L	Minimal per Litre
MTX	Methotrexate
L	Litre
MF-BIA	Multi Frequency BIA
MUAC	Middle Upper Arm Circumference
MUST	Malnutrition Universal Screening Tool
NA	Non-applicable
NB	Neuroblastoma
NDNS	National Nutrition and Diet Survey
ng	Nanogram

NG	Nasogastric
NK	Natural Killer Cell
NHL	Non-Hodgkin’s Lymphoma
NHS	National Health Service
nmol	nanomols
NR	Non-reported
NRCT	Non-Randomised clinical trial
NS	Nutrition support
NSAID	Non-steroidal Anti-inflammatory Drugs
OAD	Other Associated Diagnosis
OH	Hydroxyl
ONS	Oral Nutrition Support
OR	Odd ratio
ORAC	Oxygen Radical Absorbance Capacity
PA	Physical activity
PAL	Physical activity level
PCp	Phosphatidylcholine
PCS	Prospective Cohort Study
PEG	Percutaneous Endoscopic Gastrostomy
PEG-J	Percutaneous Endoscopic Jejunostomy
PEM	Protein Energy Malnutrition
pg	picograms
PI	Protein Intake
pmol	picomols
PN	Parenteral Nutrition
PNET	Primitive Neuroectodermal Tumours

PNRS	Simple Paediatric Nutrition Risk
PR	Protein Requirements
Pred	Prednisolone
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTH	Parathyroid hormone
PUFA	Polyunsaturated Fatty Acids
PYMS	Paediatric Yorkhill Malnutrition Score
QMU	Queen Margaret University
QoL	Quality of life
R	Radiotherapy
RBP	Retinol Binding Protein
RBW	Relative Body Weight
RC	Retrospective Cohort Study
RCPCH	Royal Colledge of Paediatrics and Childhealth
RCT	Randomised Control Trial
RDA	Recommended Daily Allowance
REE	Resting Energy Expenditure
RHSC	Royal Hospital for Sick Children
RNI	Reference Nutrient Intake
ROS	Reactive Oxygen Species
RR	Relative Risk
SANC	The Scientific Advisory Committee on Nutrition
ss-CRP	Standard sensitivity CRP
SCT	Stem Cell Transplant
SE	South East
Se	Selenium

SD	Standard Deviation
SES	Socioeconomic Status
SOD	Superoxide Dismutase
SF-BIA	Single Frequency BIA
SGNA	Subjective Global Nutritional Assessment
SGA	Subjective Global Assessments
Sig	Significant
SIGN	Scottish Intercollegiate Guidelines Network
SIMD	Standard Index of Multiple Deprivation
SNS	Sympathetic Nervous System
ST	Solid Tumours
STAMP	Screening Tool for Assessment of Malnutrition in Paediatrics
STRONG Kids	The Screening tool for Risk on Nutritional status and Growth
STS	Soft Tissue Sarcoma
T	Time of measurement
TAC	Total Antioxidant Capacity
TAS	Total Antioxidant Status
TBARS	Thiobarbituric Acid Reactive Substances
TBW	Total body water
TCh	Total Cholesterol
TEAC	Trolox Equivalent Antioxidant Capacity
TG	Triglycerides
TEE	Total Energy Expenditure
TEI	Total Energy Intake
TEM	Technical Error of Measurement
TEM%	Percentage of TEM

TER	Total Energy Requirements
TNF	Tumor necrosis factor
TSF	Triceps Skin Fold
UAFA	Upper Arm Fat Area
UAMA	Upper Arm Muscle Area
UK	United Kingdom
W	Weight
WFA	Weight for Age
WFH	Weight for Height
WHO	World Health Organisation
y	year
Zn	Zinc
µg/L	Micrograms per Litre
µmol/L	Micromole per Litre
X^2	Chi-square test
1,25(OH) D	1,25-dihydroxyvitamin D
25(OH) D	Vitamin D/ 25-hydroxyvitamin D_2

List of publications

Original articles

Revuelta Iniesta R, Wilson DC, Brougham MFH, Smail F, Davidson I, McKenzie JM. Assessment of plasma antioxidants, oxidative stress and polyunsaturated fatty acids in paediatric cancer patients: a prospective cohort pilot study. *EC Nutrition.* 2.3; 412-425.

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Experimental Biology, San Diego 2014. C293. Poster

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Revuelta Iniesta R, Wilson DC & McKenzie JM. Antioxidants and fatty acids status of paediatric cancer patients: a narrative review.

Revuelta-Iniesta R, Paciarotti I, Brougham MFH, Chin R, Brand C, McKenzie JM & Wilson DC. Plasma 25-hydroxycholecalciferol before and after supplementation in paediatric oncology patients from Scotland: a time series cross-sectional study.

Revuelta-Iniesta R, Paciarotti I, Brougham MFH, McKenzie JM & Wilson DC. Nutritional status of paediatric cancer patients from Scotland: a prospective cohort study

CHAPTER I

1. REVIEW OF THE LITERATURE

1.1 INTRODUCTION TO PAEDIATRIC CANCER

1.1.2 Paediatric cancer

The term paediatrics originates from the Greek words “paedion” (child) and iatriki (medicine), which refers to a specialty of medicine that focuses on the care of infants, children and adolescents (Lissauer and Clayden 2007). However, the age range differs from place to place; the countries from the European Union define paediatrics as children from birth up to 18 years, whilst in the USA and Canada it is from birth up to 21 years. Thus, paediatric oncology in the UK includes those children under the age of 18 who are diagnosed with benign tumours or malignant neoplasms; also referred to as childhood cancer (Pinkerton, Plowman and Pieters 2004).

Neoplasm refers to a large group of diseases characterised by unregulated and often rapid cell growth. There are three types of neoplasm: benign tumours, pre-cancer (or carcinoma in situ) and malignant neoplasm (or cancer). Benign tumours are characterised by being cohesive, expansible and localised. In contrast, malignant neoplasms are poorly circumscribed and have the ability to metastasise to both nearby and distant parts of the body via the blood system, lymphatic system and/or directly from the tumour. Finally, pre-cancer (carcinoma in situ) is an early form of localised tumour, which has the potential to become malignant (Boon, et al. 2006). Although these definitions apply to both adult and paediatric cancers, the latter exhibit a great diversity of histological type and anatomical site, in contrast to cancers seen in adults from the western world, which are mainly classified by topography only (WHO 2014). Additionally, common adult cancers, apart from leukaemia or lymphoma, are extremely rare in children (Stiller 2004).

For research purposes paediatric cancer is classified according to the International Classification of Childhood Cancer (ICCC-3) (Steliarova-Foucher et al. 2005) (appendix I). The ICCC-3 is based on morphology of the neoplasms and its aim is to standardise international population-based epidemiological studies and cancer registries to ensure international data is comparable in the field of paediatric

oncology/haematology. There are 12 main diagnostic groups and 47 subgroups. The 12 main diagnostic groups are: leukaemias, lymphomas, sympathetic nervous system tumours, brain and spinal tumours, retinoblastoma, renal tumours, hepatic tumours, malignant bone tumours, soft tissue sarcomas, gonadal and germ cell tumours, epithelial tumours and unspecified malignant neoplasm (Steliarova-Foucher et al. 2005). Following on from this classification, the different paediatric cancers may be then classified into four larger groups, allowing researchers to perform statistical analysis in studies that include smaller sample sizes. The groups are: (i) Haematological malignancies; (ii) solid tumours; (iii) brain tumours and, (iv) benign tumours.

(i) Haematological malignancies include all cancers of the blood, lymphatic system and bone marrow. Leukaemia, the most prevalent type of haematological malignancy (Cancer Research UK 2012b), is a group of malignant disorders that affect the haematopoietic stem cell compartment leading to an abnormal increase of immature white blood cells that cause damage and death by squeezing and forcing normal cells out of the bone marrow and/or by metastasising to other organs (Brooker 1994). Acute lymphoblastic leukaemia (ALL). acute myeloid leukaemia (AML) and chronic myeloid leukaemia (CML) are the three most common types of leukaemias seen in children (Stiller 2004). The main differences between these three types of leukaemia lies in the type of white blood cells affected and the speed at which the immature or malignant cells are synthesised (Pinkerton, Plowman and Pieters 2004).

(ii) Solid tumours include a large group of malignant neoplasms located in any part of the body apart from the brain (and blood, lymphatics and bone marrow). The most common are the following: lymphoma, neuroblastoma and soft tissue sarcoma (Stiller 2004, Cancer Research UK 2012b). Although lymphoma, like leukaemia, affects white blood cells (lymphoid cells), particularly B-cells and T-cells at different stages of differentiation, it starts as a solid tumour of the lymph nodes spreading rapidly into the bone marrow. For that reason, it is generally classified as solid tumour instead of a haematological malignancy (Pinkerton, Plowman and Pieters 2004). The most common types of lymphomas in children are Hodgkin and non-Hodgkin's lymphoma. Neuroblastoma is a malignant neoplasm that arises from the

sympathetic central nervous system (CNS) presenting most frequently in the adrenal gland, the paravertebral retroperitoneum, the posterior mediastinum, the pelvis and the cervical area (Pearson and Pinkerton 2004). Finally, soft tissue sarcomas are a group of heterogeneous malignant neoplasm, which involve the connective and supportive tissues (Carli et al. 2004).

(iii) Brain tumours are also malignant neoplasms originated in the CNS and located in the intra-cranium or spinal canal (Kumar, Abbas & Fausto 2006). This group of tumours can affect many parts of the brain including, the cranial nerves, the meninges, the skull, the pituitary gland and the pineal gland. They can also spread to the lymphatic tissue or the circulatory system (Kumar, Abbas & Fausto 2006). They are considered life threatening not only for their metastatic character, but also for the limited space in which they grow, obstructing other parts of the brain. In turn, this can lead to serious complications such as intracranial hypertension and epileptic seizures in the short term and cognitive impairment and death in the long term (Colledge, Walker & Ralston 2010). An example of brain tumours includes high grade glioma.

Finally, (iv) benign tumours originate in any part of the body; however, the most common site is the brain and the most common type of benign tumour is low grade glioma (Hargrave, Messahel & Plowman 2004).

1.1.2 Epidemiology of paediatric cancer

Cancer is the most common disease-related cause of childhood death in the Western World (Cancer Research UK 2012b). However, most children with cancer live in low- and middle-income countries accounting for 94% of all deaths in people aged 0-14 years (Pritchard-Jones et al. 2013). In the UK, the mean incidence of childhood cancers between 2008 and 2010 was 1603 new cases per year. Of these, 55% (883) were boys and 45% (720) were girls, whilst in Scotland alone the mean incidence was 117 per year; 53% (62) were boys and 47% (55) girls (Cancer Research UK 2012a).

Figure 1.1 shows the UK incidence of paediatric cancer according to ICCC-3. The four most common types of cancer diagnosed in the UK are Leukaemias, which

account for nearly one third (31%) of all cases, followed by brain and central nervous system (CNS) tumours (25%). Lymphomas and soft tissue sarcomas are the third and fourth most common cancers representing 10% and 7% respectively (Cancer Research UK 2012a).

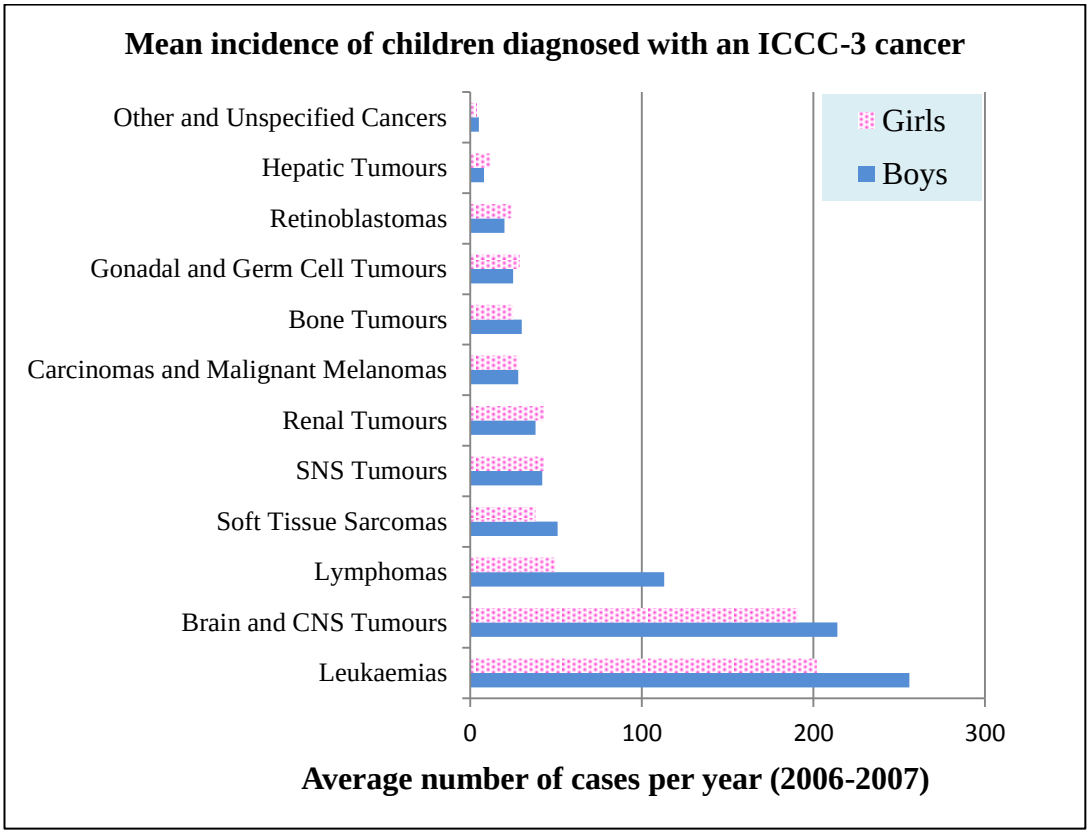

Figure 1.1 Incidence of paediatric cancer classified according to ICCC-3 in the UK (years 2006-2007) (Cancer Research UK 2012a)**.**

SNS Tumours: Sympathetic Nervous System Tumours.

Survival rates have improved considerably in recent years (figure 1.2). In the late 1960s, only 30% of children survived 5-year post-diagnosis; whilst between the years 2005-2007, the incidence improved to 80% (Cancer Research UK 2012b). Ten-year survival rates are similar to the 5-year, as 73% of childhood cancers survivors are expected to survive for 10 years (Cancer Research UK 2012b). Most survivors of childhood cancer will be cured; however, this population experience higher mortality

rates beyond 25 years from diagnosis than the general population as a result of treatment side-effects (Cancer Research UK 2012b) such as the metabolic syndrome, cardiac complications and second cancers as well as reduced bone mass density (Wallace et al. 2013, Brougham et al. 2002).

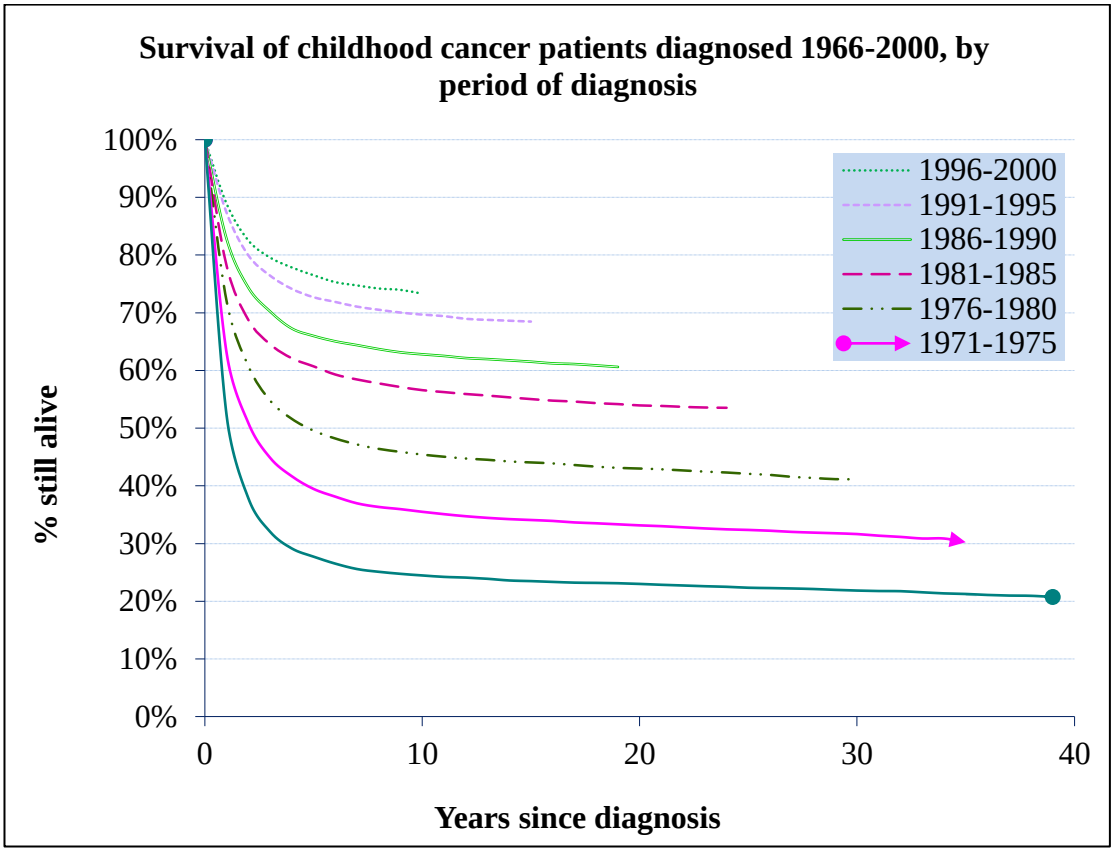

Figure 1.2 Childhood cancer survival trends of patients diagnosed between 1966 and 2000 (Cancer Research UK 2012)

1.1.3 Treatment and management of paediatric cancer

The treatment and management of paediatric cancer is complex, constantly developing and being revised (Mycroft 2010). Over the last 30 years, great advances have been made not only in the treatment of paediatric cancer, but also in the development of supportive care. This is due to new sophisticated technology, our better understanding of genetics, the success of medical clinical trials (Mycroft 2010)

as well as, the adoption of a more holistic approach to patient care with the employment of a multidisciplinary team (Pinkerton, Plowman and Pieters 2004). As the earlier epidemiological data shows all of these mean that in most cases remission and cure is achieved (Cancer Research UK 2012b, Wallace et al. 2013, Sposto 2004). Although, more intensive treatment regimens have improved survival, there is a trade off with an associated increase in treatment toxicity, which leads to higher morbidity during and after therapy. Thus the new objectives of paediatric cancer treatment are to reduce morbidity of treatment while achieving cure.

The main available cancer treatments at present include chemotherapy, radiotherapy and surgery (Boon et al. 2006). The use of both proton therapy and bone marrow stem cell transplants have recently become more popular and effective (Pinkerton, Plowman and Pieters 2004). Increasingly, treatments are being used in combination as part of clinical trials or standard regimes. This is particularly the case when the treatment aim is curative and the type of paediatric cancer is prevalent (Boon et al. 2006, Sposto 2004).

Clinical trials are designed to treat specific types of paediatric cancer and to assess the efficacy of one form of treatment or combinations of different treatments in comparison to standard regimes (Pinkerton, Plowman and Pieters 2004). Clinical trials are available for the most common type of paediatric cancers and, in Scotland; they are conducted through the United Kingdom Children's Cancer Study Group (Sposto 2004). The adjustment of therapy will be determined by many factors including; severity of disease, early response to treatment, metastasis (where applicable), age and in some cases gender (Sposto 2004, Chan 2007). Patients are stratified by risk of treatment failure (low, medium or high risk) if the diagnosis is a haematological malignancy or, by severity of disease if the diagnosis includes brain or solid tumours (stage 1, 2, 3 and 4) (Sposto 2004). Generally, the patients are categorised into groups; low intensity, medium intensity or high intensity and, consequently randomised into an arm of the trial. Clinical trials are seldom available for rare paediatric tumours, thus oncologists often refer to adult guidelines for the treatment of these cancers (Grundy and Plowman 2004).

With the recent improvement in survival rates (Cancer Research UK 2012b) and the application of more intensive therapies, which leads to higher toxicity levels and, a subsequent increase in treatment induced side-effects during and after the completion of therapy, attention is shifting to the reduction of treatment related side-effects (Sala et al. 2004). In particular, malnutrition is now regarded as a consequence of treatment and is a source of major concern since it is thought to increase the risk of morbidity (van Eys et al. 1980), mortality (Sala et al. 2004, Lange et al. 2005, Reilly et al. 1994, Butturini et al. 2007), early relapse (Viana et al. 1994) and the number of complications during the course of treatment '(van Eys 1979a; Anon.1998). Additionally, it has recently been highlighted in a SIGN guideline that long term survivors of childhood cancer might be at higher risk of developing the metabolic syndrome, cardiac complications and have a reduced peak bone mass due to related treatment side-effects (Wallace et al. 2013). The later complications may be exacerbated by malnutrition, as obesity is an independent risk factor for cardiovascular disease (Hubert et al. 1983), whilst long term undernutrition is associated with osteoporosis (Rizzoli and Bonjour 1999).

1.2 NUTRITIONAL STATUS IN PAEDIATRIC CANCER

Nutritional status is a term used to describe the body's state in relation to the consumption and utilisation of nutrients (Reilly 2008). It is assessed by comprehensively evaluating the following; anthropometry and body composition, biochemical parameters, clinical assessment and dietary intake (Thomas & Bishop 2009, Agostoni et al. 2005a). In order to understand the epidemiology, aetiology and pathophysiology of malnutrition in paediatric cancer patients, it is first essential to introduce some concepts that define children's growth and the nutritional status assessment in the general paediatric population. These concepts form the bases of this project and will be used throughout the thesis.

1.2.1 Nutritional status of children

The human body is divided into several theoretical compartments (figure 1.3). As more compartments are identified more comprehensive becomes the level of nutritional assessment; however, more advanced equipment is required for these to be measured. The simplest levels of nutritional assessment include those that assess one (body weight) or two compartments (fat free mass (FFM) and fat mass (FM)). FFM encompasses everything but FM and, can be divided into four further large compartments: visceral protein, intracellular and extracellular water and bone minerals (Norton & Olds 1996, Kyle et al. 2004a). These compartments are not static and are also affected by many factors including: growth, age and gender, physical activity, energy, micronutrient and fluids intake as well as disease.

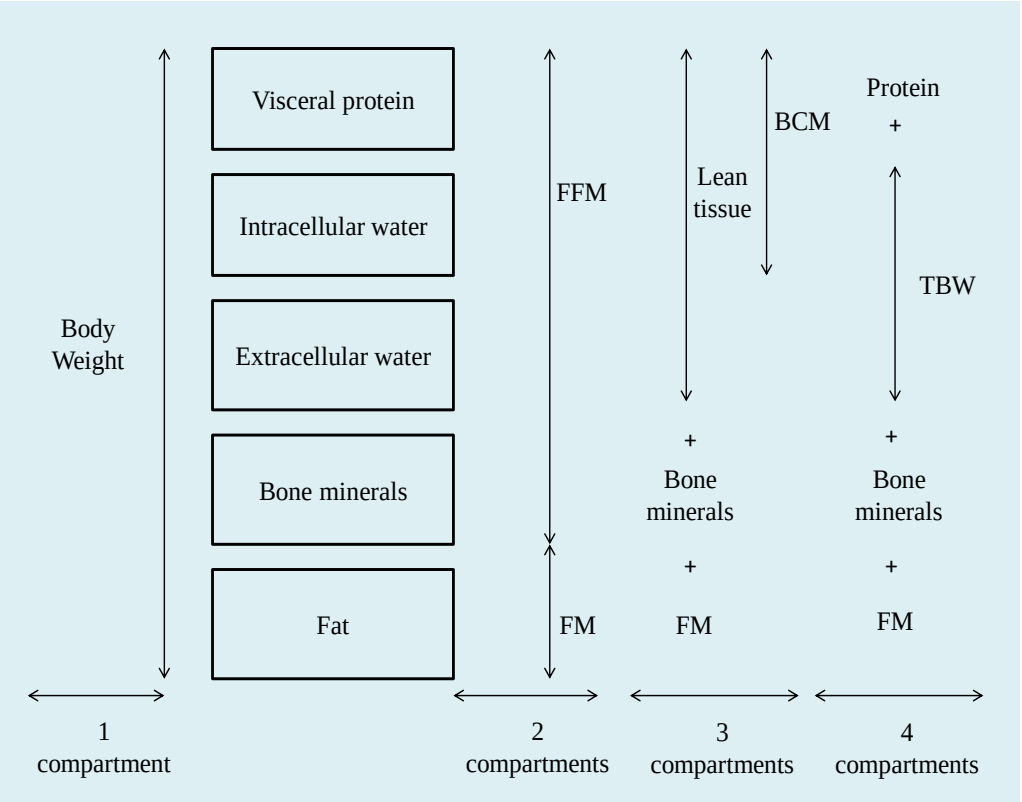

Figure 1.3 Representation of body composition and theoretical body compartments (adapted from Norton and Olds 1996, Kyle et al. 2004a)

The main difference between the nutritional status assessment of adults and children is that children grow and, are constantly changing and developing to become adults. There are three described physical phases in the growth of a child, which are accompanied by changes in body composition too: infancy (birth to up to 2 years of age), childhood (2 to 12-13 years of age) and puberty (12-13 to 18-19 years of age) (figure 1.4) (Karlberg 1989). Infancy is the shortest period of a human's life; however the rate of growth during this phase is the fastest, as most children will have doubled their height by the age of 2 and, linear growth up to this point will account for about 45% of the final height (Karlberg 1989). Infancy is also a critical period of organ and mental development; thereby severe energy deprivation during this early phase is often irreversible and may lead to both impaired physical growth and intellectual functioning later in life (Lissauer & Clayden 2007, Stoch & Smythe 1976, Frisancho et al. 1977). The childhood phase is the longest period of a child's

growth; it is slow and steady and, will account for about 40% of the final height. Finally, puberty is a period of rapid growth accounting for about 15% of the final height and characterised by changes in the sexual organs. Thus, it is the time when a child's body will change into a mature adult body (Karlberg 1989, Tanner 1990a). As children grow, changes in body composition; FFM, FM and bone minerals, also occur (Norton & Olds 1996). Subcutaneous fat will start increasing soon after birth until the age of 2 years, to progressively decrease to the lowest levels (nadir) between the ages of 6 and 8 years. After this stage, called adiposity rebound, subcutaneous fat starts to rise again until the time of the growth spurt during puberty (Tanner 1990a, Reilly et al. 2001).

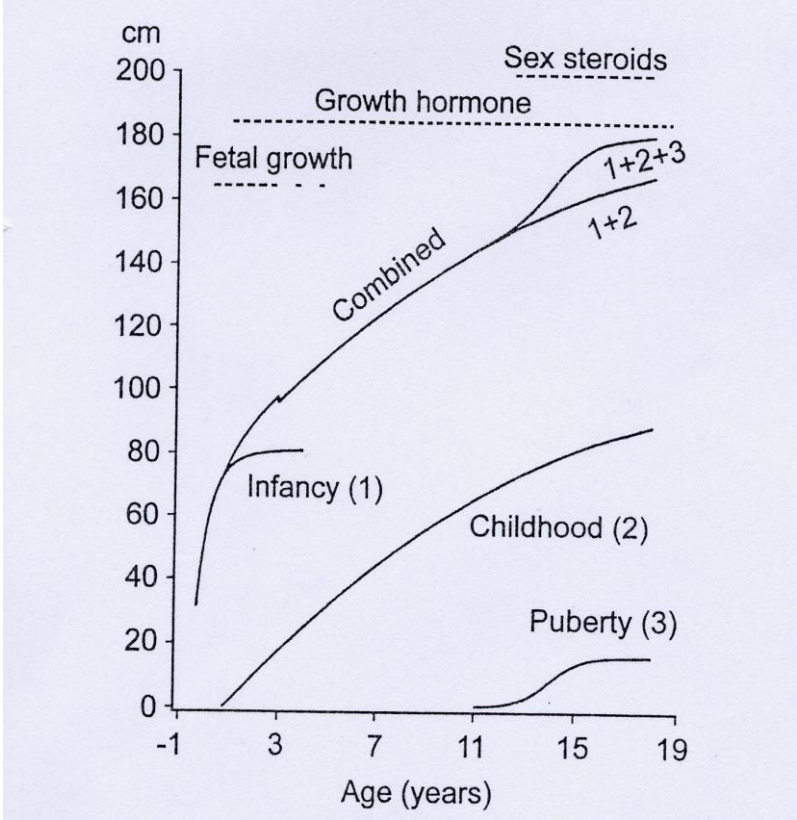

Figure 1.4 The infancy-childhood-puberty model of growth (reproduced with permission from Karlberg J, 1989)

For a child to grow optimally, dietary intake has to meet total energy expenditure (TEE) demands (Shaw and Lawson 2007). In healthy children, this includes resting

energy expenditure (REE) and physical activity (PA), which is consistent with the child's normal development. Like adults, REE also accounts for basal metabolic rate (BMR) and dietary induced thermogenesis (DIT); but in children, a percentage of the REE should be taken into consideration to allow for deposition of tissue at a rate consistent with healthy growth (Lissauer & Clayden 2007, Shaw & Lawson 2007, Butte et al. 2000).

The growth of a child can be assessed at the simplest level by using methods that provide information about one compartment only. Nevertheless, these measurements assess whether a child is growing optimally and thus are essential. Unlike adults, a standard measure is lacking (Murphy, White & Davies 2009); however, most physicians and researchers rely on national and/or international growth charts developed from population-based cross-sectional studies and longitudinal cohort studies of healthy children (Cole et al. 1995b). Growth charts illustrate growth (Cole et al. 1995b, Cole 2014) and references are expressed in a centile and standard deviation fashion. In the UK the following growth charts are available: body mass index (BMI), weight for age (WFA), weight for height (WFH), height for age (HFA), as well as head circumference (Cole et al. 1995b, de Onis et al. 2007, WHO 2007). Generally, the assessment will be based on whether the child is following its expected centile (Ahmad et al. 2014). Consequently, a single measurement will have limited value, unless centile lines fall below the 0.4th centile or above the 99.6th centile (de Onis et al. 2007, WHO 2007).

To assess whether the body has enough reserves stored in the form of FFM and FM (2 compartments), there are other simple methods that can be used, which include mid-upper circumference (MUAC), triceps skinfold thickness (TSF) (Norton & Olds, 1996, Frisancho 1981, Frisancho 1974) and bioelectrical impedance analysis (BIA) can be used to assess more than 2 compartments. At present, there are not growth charts available for MUAC and only one, which was performed in the 1960s and included healthy UK children from the age of one month to 19 years, is available for TSF measurements (Tanner & Whitehouse 1975, Oakley et al. 1977). A few authors have developed percentile ranges also obtained from populations of healthy children, from which the data can be compared against (Frisancho 1981, Frisancho 1974,

Ozturk et al. 2009, Mwangome et al. 2012). Upper arm muscle area (UAMA) and fat mass muscle area (UAFA) can be calculated from MUAC and TSF; for which, a chart (Tanner & Whitehouse 1975) and percentile ranges have also been developed (Frisancho 1981).

BIA estimates TBW, FFM and FM by passing an electric current through the body, which is conductive in lean tissue due to its high water and electrolyte content, but it is not conductive in the fat tissue (resistance). The number of body compartments that the BIA measures depend on the frequency used and the most widely used are the single and multi-frequency BIA. Single frequency (SF) BIA operates at 50 kHz and measurers 3 compartments; the weighted sum of intracellular and extracellular fluid without differentiating between these two compartments, which is defined as TBW in simple terms. From this, FFM and FM can be estimated (Kyle et al. 2004). As it assumes that the intracellular and extracellular water compartments are homogenous, its use has been reported to be more accurate in subjects with normal hydration status (Gudivaka et al. 1999). Multi frequency (MF) BIA operates using a range of frequencies between 1 and 500 kHz and measures 4 compartments as it differentiates between intracellular and extracellular fluid (Kyle et al. 2004). As a result its use has been reported to be more accurate in critically ill patients who present with shifts in fluid distribution between the intracellular and extracellular water compartments (Olde Rikkert et al. 1997).

The estimation of FFM% and FM% from BIA in adults is considered accurate and reliable; however its use in children remains controversial. The reasons for this have been attributed to the rapid changes that occur in the body composition of children, which have made difficult the development of predictive equations (Mast et al. 2002, Rowe et al. 2006, Weinand et al. 2000). Accuracy has also been argued in both undernourished (Mast et al. 2002, Weinand et al. 2000) and obese children (Wabitsch et al. 1996). Finally some authors claimed that the estimation of FFM and FM in children is more accurate when these are calculated from MUAC and TSF measurements, which has also been attributed to the rapid changes in body composition that occur in children (Mast et al. 2002, Weinand et al. 2000, Hammond

et al. 1994, Schaefer et al. 1994b). Yet, some authors report satisfactory levels of accuracy (Schaefer et al. 1994a, Okasora et al. 1999).

Nutritional status can also be assessed using other superior and more reliable methods such as Isotope-dilution, Dual-energy X-ray absorptiometry (DEXA), Magnetic Resonance, Computed Tomography, Densitometry and total body potassium (Thomas & Bishop 2009, Wabitsch et al. 1996, Barbosa-Cortés et al. 2007, Frisk et al. 2012, Inaba et al. 2012, Collins et al. 2010, Zhang et al. 2014). Nevertheless, their role is generally for research purposes and limited in the clinical setting (Brennan 1998, Brennan et al. 1997, Murphy et al. 2009).

1.2.2 Malnutrition in children

Malnutrition is defined as a state in nutrition in which an inadequate intake of energy, protein or other nutrients causes adverse effects on physiological functions, body composition and clinical outcome (Agostoni et al. 2005a, BAPEN 2010). This imbalance between energy intake and energy expenditure is expressed as undernutrition (negative energy balance) and overnutrition (positive energy balance). (Brennan 1998, Anon.2006a, BAPEN 2010). As per definition, the term malnutrition will be used to refer to both undernutrition and overnutrition. Whilst undernutrition in children is recognised when there is rapid weight loss or failure to gain weight (to enable optimal growth), poor linear growth (height) and faltering growth (or failure to thrive); overnutrition, like in adults, is recognised by clear cut off values, which are grouped into two categories: overweight and obesity.

Undernutrition in children occurs when there is not sufficient energy intake to support an increase in FFM and FM, which allows for both normal body functions and growth (Reilly 2008, Agostoni et al. 2005a). Undernutrition has been classified as acute and chronic. Acute undernutrition is the first sign of undernutrition and, normally, presents as either rapid weight loss or, poor weight gain followed by weight loss. In these cases the length or height is not generally affected. Thus, the child will have low WFH and low BMI (Agostoni et al. 2005a, Waterlow 1972). Reduced energy intake affects body stores and thus body composition, consequently an undernourished child may also present with reduced muscle mass (FFM) and

adipose stores (FM) (Brinksma et al. 2012, Brinksma et al. 2014). Chronic undernutrition is the result of long term energy deprivation, which results in poor linear growth (low HFA) (Waterlow 1972) and stunting if catch up growth becomes impossible. In infants, it may also affect mental development, which may present as a reduced head circumference (Martins et al. 2011, Cornelio-Nieto 2007). The muscle mass and adipose tissue will also be reduced if the child has acute on chronic undernutrition (Waterlow 1972).

In contrast, overnutrition occurs when energy intake exceeds energy expenditure and eventually results in an increase in FM (Brinksma et al. 2014, Anon.2006b). An overnourished child will present with high WFH, high BMI and high FM% (Reilly 2008, Waterlow 1972, Reilly 2006, Reilly, Kelly & Wilson 2010). Reduced muscle mass has been defined as protein energy malnutrition (PEM); however protein energy malnutrition might be accompanied by low fat mass (undernutrition) or high fat mass in children (Brinksma et al. 2014). In adults and the elderly, the combination of muscle wasting and high fat mass with or without the presence of a mild inflammatory response has been defined as Sarcopenic obesity and has extensively been studied (Stenholm et al. 2008, Tsai 2012). Yet, this term, unlike osteopenia (loss of bone mass) has never been defined in children.

Worldwide, the prevalence of malnutrition in children differs significantly from low (developing countries) and high income countries (developed countries) (Stephenson et al. 2000, de Onis et al. 2010, de Onis et al. 2004). Undernutrition in developing countries is thought to affect 19% to 30% of children; whilst in developed countries, it is estimated to affect only 1.6% (Stephenson et al. 2000, de Onis et al. 2010) and is generally associated with acute or chronic conditions (Reilly 2002). In the UK, the prevalence of undernutrition in children under 42 months of age has been estimated to be 3.3% (Armstrong et al. 2003), whilst no recent data exist from older children. In contrast, worldwide prevalence of overnutrition (overweight and obesity) is higher in developed countries and in the adolescent population (10-19 years) (de Onis et al. 2010, Bibiloni et al. 2013). Worldwide, the prevalence of overweight and obesity has been explored in preschool children (less than 5 years of age) and adolescents (10-19

years) and, data have been reported separately. Overnutrition in preschool children is estimated to be 7% with higher prevalence rates reported from developed countries (12%) than developing countries (6%) (de Onis et al. 2010). Higher prevalence of overweight and obesity have been reported in the adolescent group, whereby prevalence ranges from rates as low as 5% in China to as high as 30% in North America (de Onis et al. 2010, Bibiloni et al. 2013). The UK appeared to have one of the highest prevalence of overnutrition with 33% of boys and 34% of girls being overweight or obese. Prevalence of overweight is 15% for both boys and girls and obesity is 18% for boys and 19% for girls (Bates et al. 2011).

1.2.3 Nutritional status of paediatric cancer patients

The nutritional status of paediatric cancer patients has been studied for a long time and malnutrition, in particular undernutrition, in this population has long been recognised (van Eys 1979a, Sala et al. 2012, Jaime-Pérez et al. 2008); yet, its management remains variable (Sala et al. 2004, Brinksma et al. 2012, Reilly et al. 1999), with many malnourished children going unrecognised and consequently untreated (Antillón et al. 2008). Until now, the exact prevalence of malnutrition has not been determined and the point during the natural history of the cancer disease (diagnosis, during treatment or after the completion of therapy) when this occurs is not known. However, prevalence is known to differ in low and high income countries (Sala et al. 2004, Barr et al. 2008, Sala et al. 2005, Sala et al. 2008). The prevalence of undernutrition in low income countries may average 50%, whilst prevalence of undernutrition in high income countries is thought to be more associated with the type of tumour and the severity of the disease. For instance, undernutrition has been reported to be more prevalent in children diagnosed with Neuroblastoma, Wilms tumour and Ewing's sarcoma (Sala et al. 2004).

In contrast, the prevalence of obesity of paediatric cancer patients has not been studied as extensively, perhaps because this is a relatively new global health issue. One study from Mexico reported that 24% of patients diagnosed with ALL were either at risk of becoming overweight or were already overweight at diagnosis (Jaime-Pérez et al. 2008); whilst more evidence exists in high income countries

where prevalence of obesity have been reported in children with ALL (Gofman & Ducore 2009, Baillargeon et al. 2005, Baillargeon et al. 2006, Baillargeon et al. 2007, Odame et al. 1994, Reilly et al. 1999) and in survivors of childhood cancer (Oeffinger et al. 2003, Hudson et al. 2003), especially those diagnosed with ALL.

To date, no prospective study investigating the nutritional status of paediatric cancer including patients at different stages of the disease have been performed in Scotland. Phase I of this project therefore represents the first data that we have and the results have shown a pattern in which children diagnosed with a solid tumour tend to become undernourished, whilst those with a haematological malignancy tend to become obese. Moreover, the prevalence of undernutrition and overnutrition was significantly higher than healthy children from the UK when the data was stratified into these two categories only (solid tumours and haematological malignancies) (Paciarotti I 2013). Given the small size of the cohort investigated in this study, caution needs to be exercised when drawing these initial conclusions.

At the end of phase I there was data available for 26 patients. Nutritional status had been assessed using BMI centiles (primary outcome), MUAC, TSF and BIA at diagnosis, baseline and during different phases of treatment (3, 6, 9, 12 and 18 months). The study classified the cancer diagnosis into two categories; solid tumours and haematological malignancies. When BMI was used to assess nutritional status, the study revealed that children diagnosed with solid tumours had the highest prevalence of undernutrition (27% (3/11) at diagnosis, 25% (4/16) at baseline and 17% (2/12) at 3 months) whilst no child diagnosed with a haematological malignancy was undernourished at any stage. In contrast, children diagnosed with haematological malignancies had the highest prevalence of obesity at 3 months (50% (4/16)) and 6 months (50% (2/4)). In addition, the study showed that the solid tumour group had lower TSF, MUAC, UAMA and UAFA values at diagnosis than the haematological malignancy group and this was more exaggerated at 3 months. Thus, children diagnosed with solid tumours as well as been more likely to be undernourished appeared to have a lower muscle and fat mass. In contrast, children diagnosed with haematological malignancies showed fat accumulation and were not depleted (low FFM) (Paciarotti I 2013).

On the account of findings from phase I, the absence of a published systematic review which investigates the worldwide prevalence of undernutrition and overnutrition at different stages of the disease and possible associations between malnutrition and clinical outcomes, a systematic review which investigates these topics was planned at the start of phase II. This will be discussed in chapter II, section 2.1.

1.3 AETIOLOGY AND PATHOPHYSIOLOGY OF MALNUTRITION IN PAEDIATRIC CANCER

The aetiology and pathophysiology of malnutrition in paediatric cancer is multifactorial and complicated. It is characterised by either a reduction or an increase in dietary intake along with metabolic and hormonal changes, which at present are not entirely understood. The different factors at play in the pathogenesis of malnutrition in cancer can be divided into three broad groups: (1) the disease and its natural history; (2) the various treatment modalities (surgical and medical) and their effects on the individual's nutritional status and, (3) the social and psychological influences of the cancer and its associated treatment on the child and its family.

1.3.1 Aetiology and pathogenesis of malnutrition: cancer and its natural history

Cancer induces a common immune response which differs in severity and has wide ranging metabolic effects (Seyfried & Shelton 2010). The progression of the cancer and its effects on nutritional status is dependent on many factors; most importantly the type, stage and location of the tumour, but also the gender, age and genotype of the host (Sala et al. 2004, Ross 2007). Although, to date there has been limited research into the various factors contributing to the development of malnutrition in paediatric cancer patients, numerous studies have been performed in animals and the adult population (Sala et al. 2004, Brinksma et al. 2012, Gough et al. 1996). It is now understood that this "common inflammatory response" is mediated by the release of cytokines, including interleukins, interferon (IFN) and tumour necrosis factor (TNF), which is initiated by both host and tumour cells (Bauer et al. 2011). Cytokine receptors, which produce metabolic changes when stimulated, have been found in the hypothalamus (Bauer et al. 2011), muscle, and adipose tissue (Gough et al. 1996). Once activated, these induce anorexia, which in turn leads to a reduction in dietary intake, skeletal muscle catabolism and to a lesser extent lipolysis (see figure 1.5).

Additionally, the synthesis of acute phase proteins, such as CRP and ferritin, in the liver is up regulated, muscle protein synthesis is down regulated and muscle catabolism is up regulated for use as "quick" energy source (Ritchie et al. 1999). All of these results in weight loss, particularly loss of FFM (Picton 1998). In adults with advanced disease, this can lead to cancer cachexia, which is defined as an irreversible

hypermetabolic state in which progressive weight loss, muscle atrophy, loss of physical function and lethargy occur secondary to malignancy (Fearon et al. 2011). However, childhood cancer cachexia has not been defined and, studies reporting prevalence of undernutrition in this population do not report cancer cachexia; instead prevalence of undernutrition, severe undernutrition and PEM are reported in both low and high income countries (Brinksma et al. 2012, Sala et al. 2012, Elhasid et al. 1999, Israels et al. 2010).

Data investigating the degree of inflammation at the time of diagnosis and its relationship with nutritional status in paediatric cancer patients is limited. The type of tumours most commonly associated with undernutrition at diagnosis are neuroblastoma, Wilms tumour and Ewing's sarcoma (Sala et al. 2012, Israels et al. 2010, Green et al. 2008); however, as reported by a recent systematic review (Brinksma et al. 2012), no study has investigated the relationship between inflammation and malnutrition in these tumours at the time of diagnosis. Two studies have been found to investigate this in children diagnosed with ALL (Siimes et al. 1991) and unspecified types of leukaemias (Yu et al. 1994). Siimmes et al (1991) measured TNF levels in a cohort of 12 children compared to 25 healthy controls and, found that although TNF levels were high at diagnosis; this was not correlated with muscle and fat mass. Similarly, Yu et al (1994) investigated CRP levels in 25 children with leukaemia; 6 were newly diagnosed, 3 had relapsed and 16 were in remission and on treatment; the type of leukaemia was not specified. CRP levels were higher in newly diagnosed and relapsed patients, who were not on treatment, than in those who were on remission and on treatment. Again, none of the children were undernourished at diagnosis, which was attributed to the early detection of the cancer. Of note, both studies included very small sample sizes, which might have contributed to their findings.

A further two studies have solely investigated inflammation in cohorts of paediatric cancer at the time of their diagnosis; yet nutritional status was not measured (Wieland et al. 2003, Giordano et al. 2010). Giordano et al (2010) found higher levels of inflammatory markers (IL-6 and TNF) in newly diagnosed ALL children when compared with both healthy controls and those who were receiving treatment.

Wieland et al (2003) meanwhile measured CRP levels in 95 children with newly diagnosed Hodgkin's lymphoma and 6 healthy controls. Higher CRP levels correlated with disease stage, whereby those children with stage I Hodgkin's lymphoma had the lowest CRP levels.

In conclusion, the small body of evidence presently available raises the possibility that children diagnosed with leukaemia and Hodgkin's lymphoma have an increased inflammatory response at the time of diagnosis. The evidence for an association with undernutrition does not yet exist. However, the most important conclusion we can draw is that there remains a lot of work to be done investigating the hypothesis that an inflammatory response is driving undernutrition as has been demonstrated in adults with malignancy. In particular future studies need focus on the malignancies most commonly associated with undernutrition.

In adults, it has been proposed that some individuals have an increased susceptibility to developing cachexia due to genetic polymorphisms. Important proteins have been identified to be involved in the mediation of the previously described inflammatory reaction, but also the regulation of appetite (and therefore energy intake) and the metabolism and utilisation of nutrients. It is thus held that the host genotype may also contribute to some of the differences seen in the nutritional status of paediatric cancer patients at diagnosis (Ross 2007, Picton 1998).

Many studies performed in adults who have an increased susceptibility to develop cancer cachexia have shown that these tend to have genetic polymorphisms of specific genes that code for the synthesis of inflammatory markers, thus increasing the inflammatory response (Zhang et al. 2007, Solheim et al. 2011, Tan and Fearon 2010). Equally, many genes have been identified which play a role in the regulation of appetite. For example, the expression of the Agouiti-related protein (AGRP) in the hypothalamus is thought to stimulate appetite, thereby leading to an increased food intake. In normal circumstances, the action of leptin suppresses AGRP expression and results in a reduction of food intake. However, in some individuals an overexpression of AGRP has been associated with obesity (Rossi et al. 1998, Schwartz et al. 1997), whilst under-expression has been associated with anorexia

nervosa (Vink et al. 2001). Numerous genes involved in the regulation of TEE have been described; with some polymorphisms been shown to reduce TEE, thus making individuals more susceptible to becoming obese (Yang et al. 2003, Feldman and Hegele 2000), and others been shown to increase the release of energy and consequently the TEE, thereby leading to weight loss (Astrup et al. 1999). Finally, polymorphism of the TNF alpha-308 G/A promoter, which plays a role in lipolysis and lipogenesis, have been found to be associated with excess body fat in women (though not in men) (Hoffstedt et al. 2000). Nevertheless, to date, no studies have been undertaken in paediatric cancer patients and thus the potential role of host genetic polymorphism on nutritional status remains unknown.

The final factor to consider, which may lead to malnutrition, is some of the innate characteristics of the tumour. Already we have seen that tumour type may well prove to be associated with risk of malnutrition, but it is already accepted that the location and size of the tumour can lead to malnutrition. For example, brain tumours, such as craniopharyngioma, are known to have the potential to cause raised intracranial pressure. In infants this presents with an increasing head circumference, vomiting and poor feeding, leading to failure to thrive (Hargrave, Messahel & Plowman PN 2004). In older children it can present with progressive characteristic headaches (worse in the morning and aggravated by posture) which are associated with vomiting, with focal neurological deficits and more acutely with seizures or altered conscious levels. Another example, relatively common in children, is Multisystem Langerhans cell histiocytosis (LCH), which may present with gastrointestinal involvement of the mucosa and submucosa of the small and large intestine. In these cases, the child will have severe malabsorption; which if not treated will lead to rapid weight loss and eventually failure to thrive and, chronic diarrhoea with protein losing enteropathy causing PEM (Gadner & Grois 2004). Obstruction of the gastrointestinal tract may occur in cancers affecting the oesophagus, such as in children diagnosed with large nasopharyngeal carcinomas (Grundy & Plowman 2004). In this case the child will either be in pain when trying to swallow or will not be able to swallow at all, thus reducing dietary intake.

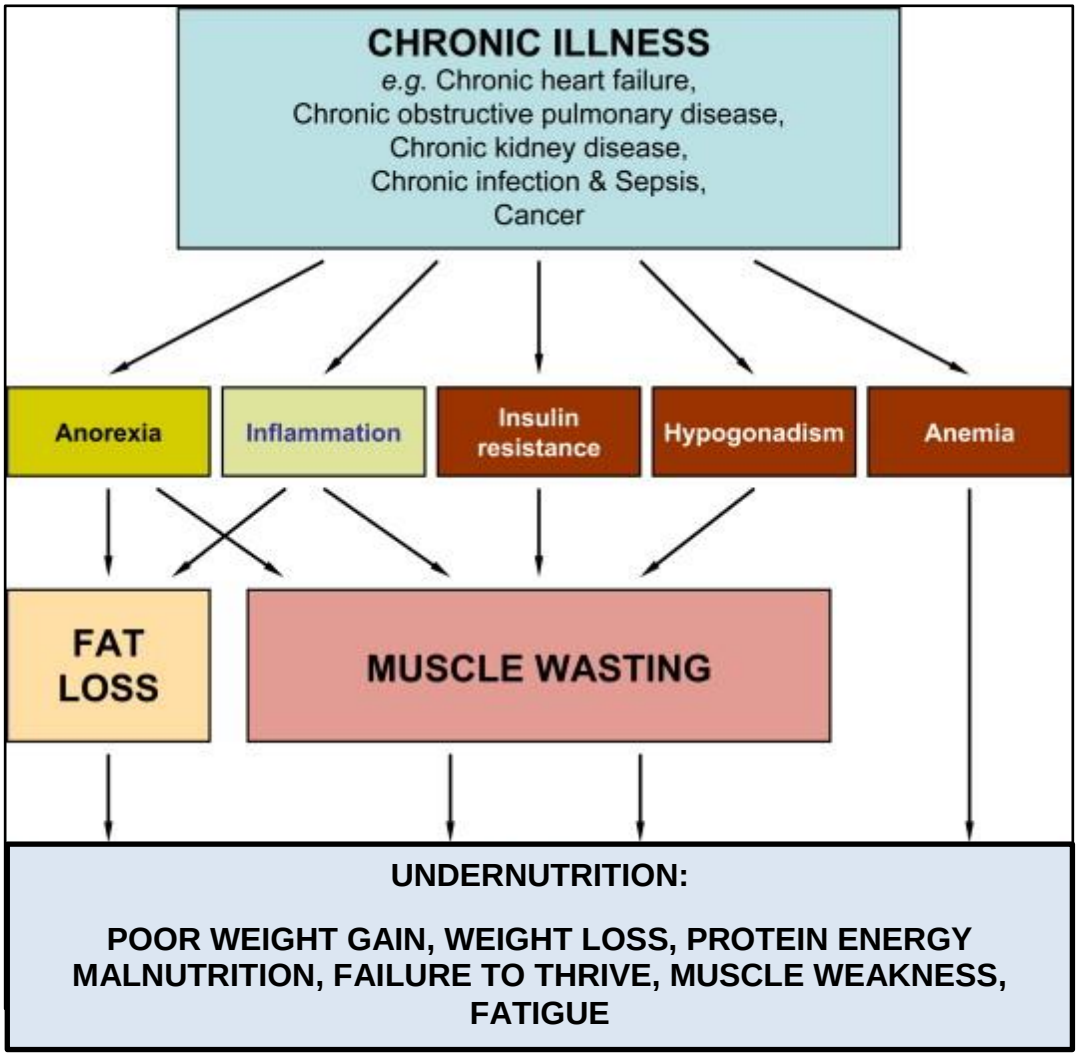

Figure 1.5 Conceptual framework of causes of undernutrition. Model adapted from Evans and co-workers (Evans et al. 2008)**.**

1.3.2 Treatment modalities: effects on nutritional status

As has been previously mentioned, management protocols designed with curative intent are used in the management of childhood cancer and involve the concurrent use of a variety of treatment modalities. At present the primary available treatments include chemotherapy including immunosuppressants, radiotherapy and surgical interventions. Additionally, an increasing number of children are using complementary and alternative therapies to help reducing treatment induced-side effects (Bishop et al. 2010), which may themselves not be relieved by conventional medicine (Spix 2008). Thus patients are exposed to multiple agents and therapies

often acting synergistically which together have wide reaching effects on their nutritional status (Boon et al. 2006, Donaldson 1982).

1.3.2.1 Chemotherapy

Cytotoxic drugs work by targeting rapidly dividing cells and essentially killing them. They have both anti-cancer activity and the potential to cause acute and long term damage to normal tissues (BNF 2009). There are four main classes of cytotoxic drugs that are used in the treatment of paediatric cancer, which include: alkylating drugs, cytotoxic antibiotics, antimetabolites (vinca alkaloids and etoposides) and, other antioneoplastic drugs (BNF 2009). Although their mode of actions differ, some of their side-effects are common to all drugs and, their effect on the nutritional status of children will depend on the following factors: (i) the intensity of the treatment, (ii) whether the child is hypermetabolic while receiving treatment and, (iii) the individual phenotype's ability to metabolise and excrete active drug metabolites (Buxton & Benet 2011, Lennard 1999). Some of the most common side effects of chemotherapy agents associated with malnutrition are presented in table 1.1.

Table 1-1 Most common side-effects associated to chemotherapy agents that may lead to malnutrition (adapted from Donaldson 1982; BNF 2009)

Side-effects	Drugs
Anorexia, nausea, vomiting	Adriamycin 5-Azacytidine c/s-Diamminedichloroplatinum Cyclophosphamide Nitrogen mustard Procarbazine
Mucositis	Actinomycin D Adriamycin Bleomycin Daunomycin 5-Fluorouracil Methotrexate Vinblastine
Diarrhoea	Actinomycin D 5-Azacytidine 5-Fluorouracil Methotrexate
Constipation and adynamic ileus	Vinblastine Vincristine
Taste and smell changes	Allopurinol Carboplatin Doxorubicin Methotraxate
Liver impairment	Asparaginase Azaserine Methotrexate 6-Mercaptopurine Mithramycin
Pancreatitis	Asparaginase
Renal impairment	Retinoic acid Ifosfamide Allopurinol Methotrexate Etoposide
Peripheral neuropathy	Vincristine Vinblastine Clofarabine Fludarabine Phosphate

(i) Intensity of the treatment

Generally, the more intensive and frequent the treatment the more of the above listed side effects the host will have, thus increasing the risk of undernutrition. If patients are not adequately treated with nutrition, fluid and electrolytes there is the possibility of severe weight loss, dehydration and death (Pinkerton, Plowman & Pieters 2004, van Eys 1979a, Donaldson 1982, van Eys 1986, van Eys 1979b). The most common side effect is mucositis. This occurs when the epithelial cells, which make up the mucosa of the oropharynx and small intestine, are damaged resulting in inflammation and ulceration. Clinically this presents with pain and bleeding in the mouth and throat and diarrhoea. The latter results in malabsorption of micro and macronutrients, fluid and electrolyte imbalances, and together mucositis can cause temporary gastrointestinal failure in severe cases (Bauer et al. 2011, Donaldson 1982, Matthay et al. 1999).

A serious problem can arise when liver and or renal impairment is precipitated by the use of an intensive regimen and or the use of specific hepatotoxic or nephrotoxic agents (Matthay et al. 1999). This is because most of the cytotoxic agents are metabolised in the liver and excreted in the urine through the kidneys (Buxton & Benet 2011). Thus when impaired, there is a resultant accumulation of these cytotoxic agents in the blood stream with the risk of more severe side effects, not to mention the other sequelae of renal and hepatic failure; uraemia and elevated ammonia levels, which taken together exacerbate the risk of undernutrition (Colledge, Walker & Ralston 2010).

(ii) Is the child hypermetabolic while receiving treatment?

There is limited data investigating the effects of treatment on inflammatory response, macronutrients metabolism and total energy expenditure in paediatric cancer patients. An increase in total energy expenditure due to tumour burden, inflammatory response and chemotherapy treatment has been reported in adults with advanced disease (Solheim et al. 2011, Tisdale 1997). In children the data is contradictory, as some studies report an increase in REE (Kien and Camitta 1987, Schmid et al. 2005), others report no change (Green et al. 2008, Bond et al. 1992, Delbecque-Boussard et al. 1997, den Broeder et al. 2001, Vaisman et al. 1993) and others report a decrease

in REE; all studies were performed while the patients were receiving treatment (Duggan et al. 2003). Additionally, most studies found that children at diagnosis, before any form of treatment was given, had higher metabolic rates than after the commencement of treatment (Kien and Camitta 1987, Schmid et al. 2005, den Broeder et al. 2001, Stallings et al. 1989), thereby indicating that children at diagnosis might be hypermetabolic and that this may gradually reduce with the initiation of chemotherapy. This has been attributed to a decrease in FFM (Duggan et al. 2003), but also to the inhibition of the inflammatory response originally caused by the tumour (Yu et al. 1994). A few cautionary notes must be considered, most studies were performed in children with ALL (Kien & Camitta 1987, Schmid et al. 2005, Bond et al. 1992, Delbecque-Boussard et al. 1997, Vaisman et al. 1993, Duggan et al. 2003, Stallings et al. 1989), only two studies investigated children diagnosed with other cancers; neuroblastoma (Green et al. 2008) and solid tumours (den Broeder et al. 2001) and, one study not only included children with any cancer (ICCC-3), but also children diagnosed with aplastic anaemia; all undergoing Haematopoietic stem cell transplantation (HSCT) (Duggan et al. 2003). Moreover, all studies were small in sample size (n=8-26), probably due to the difficulty in measuring REE in children with cancer (Brinksma et al. 2012). Thus, due to these limitations (small sample sizes and the heterogeneity of the populations studied), it remains unclear whether children at the time of cancer diagnosis are hypermetabolic; nevertheless, it appears that most children during cancer treatment are not. Therefore, more research is warranted looking at whether children are hypermetabolic at different stages of the disease, in order to understand the nutritional status of paediatric cancer patients.

(iii) Individual phenotype variability

The author has previously considered the concept of host susceptibility to malnutrition following the discovery of multiple genetic polymorphisms involved in the regulation of inflammation, metabolism and utilisation of nutrients (Picton 1998; Ross 2007). More widely genetic polymorphisms of enzymes and transporters involved in drug metabolism, which result in altered protein expression and activity, have been well described (Lee et al. 2005, Gervasini and Vagace 2012). As part of this expanding field of biomedical research several genetic polymorphism have been

identified which play a role in the pharmacokinetics and pharmacodynamics of chemotherapy drugs (Lee et al. 2005, Riddick et al. 2005). It has also been observed that there are significant genetic differences between different ethnic groups (Lee et al. 2005). The key message here is that we are beginning to understand why different patients respond so differently to treatment with some experiencing more toxicity than others (figure 1.6). This concept also goes some way to explaining the variability in nutritional status of children with similar demographic characteristics, diagnosed with the same condition and treated with the same protocol.

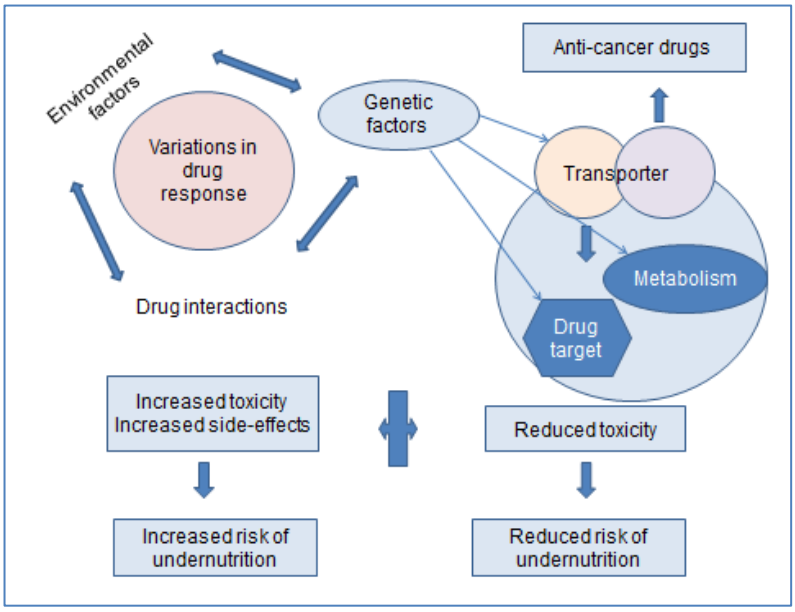

Figure 1.6 Multiple factors contributing to variations in drug response and the consequent risk of undernutrition**.** Adapted from (Lee et al. 2005)

1.3.2.2 Glucocorticoids

Glucocorticoids, in particular dexamethasone and prednisolone, alongside cytotoxic agents have long been the mainstay of treatment for ALL, Hodgkin's disease, non-Hodgkin's lymphoma, and B-Cell lymphoma (BNF 2009). They are also employed for their anti-tumour effects in the treatment of other cancers, to prevent organ transplant rejection, such as following HSCT and, to reduce intracranial pressure in patients diagnosed with brain tumours. Additionally, they are routinely used in smaller doses as an antiemetic. Although, their anti-tumour properties have long been

demonstrated to be effective, they are known to have multiple serious side-effects, including an increased risk of malnutrition, particularly overnutrition and obesity. This is detailed in table 1.2 (Odame et al. 1994, Wallace et al. 2003, Ahmed et al. 2002, Groot-Loonen et al. 1996).

Despite the well-known metabolic effects of this group of drugs, there has until now been very little work looking into the effects of glucocorticoids on the nutritional status of childhood cancer patients during and after treatment. Therefore, this is not entirely understood (Reilly et al. 2001). This dearth of research has been attributed to a number of factors: (i) the difficulty of studying the effect of a single agent since most childhood cancers are treated with multiple agents; (ii) in order to estimate the precise prevalence of undernutrition, overnutrition and obesity there is a need for large expensive multicentre cohort studies; (iii) The technical challenges involved with the necessary mechanistic studies which would allow us to investigate whether or not specific childhood cancer patients treated with glucocorticoids have a genetic predisposition for malnutrition, especially obesity (Reilly 2009b). (iv) Another reported barrier is the difficulty of performing physiological studies that investigate the relationship between TEE and energy intake and, the development of malnutrition in this population (Reilly 2009b).

Table 1-2 Most common side-effects associated to glucocorticoids that may lead to malnutrition

Effects of glucocorticoids	Side effects	Malnutrition	References
Immunodeficiency	Increase risk of infection	Undernutrition	(Dejean and Richard 2013)
Appetite stimulant (leptin resistance)	Increase appetite → increase food intake	Obesity	(Davies et al. 2004)
Changes in macronutrient metabolism	Increased proteolysis	Muscle breakdown → reduced FFM	(Schakman et al. 2009)
	Lipolysis and increase of fatty acids in circulation	Ectopic fat distribution (liver, muscle, and central adipocytes) → Obesity	(Peckett et al. 2011)
	Insulin resistance and increased gluconeogenesis	Hyperglycaemia	(McMahon et al. 1988)
Salt and water retention	Oedema	May mask undernutrition or PEM	(Motil 1998)
	Delay puberty, slow growth	Reduced HFA, stunting	(Motil 1998, Dalton et al. 2003)
Inhibition of osteoblast, stimulation of osteoclast, reduced calcium absorption, increased vitamin D catabolism	Osteopenia, rickets, osteoporosis, osteonecrosis	Reduced bone mass density → weakness Increase risk of fracture	(Zhou et al. 2006)

1.3.2.3 Radiotherapy

Radiation therapy is the medical treatment of cancer whereby ionising radiation is employed to either kill or control cancerous cells (Boon et al. 2006). Ionising radiation either damages DNA directly or indirectly by the generation of free radicals OH and H from water and reactive oxygen species from oxygen. Cells with pre-existing DNA defects like cancerous cells tend to be more sensitive to ionising radiation than healthy cells. However, healthy cells will also be affected by radiation therapy (O'Donoghue 2004). The acute side-effects of radiotherapy on nutritional status are generally well understood and predictable (O'Donoghue 2004). However,

evidence on the medium to long term effects of radiotherapy are scarce, with most research focusing on the effects of cranial radiotherapy on nutritional status (Dalton et al. 2003, Lustig et al. 2003, Müller et al. 1998, Müller et al. 2001, Armstrong et al. 2010, Siviero-Miachon et al. 2009a). The acute effects of radiotherapy depend on the following factors: the dose of radiation, the duration of treatment and the location and size of the tumour treated (O'Donoghue 2004). Patients receiving ionising therapy to the brain, head and neck and, abdominal and pelvic region will have an increased risk of malnutrition, which at the same time will be exacerbated by higher doses of radiation (Donaldson 1982, O'Donoghue 2004, Donaldson et al. 1981). This is because of the damage to the mucosa of the digestive tract, which like with chemotherapy can lead to mucositis of the oropharynx and small intestine. The resultant symptoms often render patients unable to eat and cause malabsorption of macro and micronutrients with gastrointestinal failure occurring in severe cases. Untreated dehydration, deranged electrolytes and weight loss can all result in death (Grundy & Plowman 2004, Donaldson 1982, O'Donoghue 2004). It should be remembered though that head and neck cancers as well as tumours of the gastrointestinal tract are rare in children (Grundy & Plowman 2004).

There is a lot of evidence to show that the use of cranial irradiation in children treated for ALL and brain tumours may lead to obesity later in life (Dalton et al. 2003, Lustig et al. 2003, Müller et al. 1998, Müller et al. 2001, Armstrong et al. 2010, Siviero-Miachon et al. 2009a). Currently, with the introduction of new protocols, the use of this form of therapy is limited to some children with refractory ALL or with optical nerve infiltration (Sposto 2004); however, alongside surgery it is the main form of treatment for craniopharyngioma (Hargrave, Messahel & Plowman 2004).

1.3.2.4 Surgery and haematopoietic stem cell transplantation

Surgery is another common form of treatment that can contribute to the pathophysiology of malnutrition. It is well known that surgical trauma causes an increase in resting energy expenditure as a result of inflammation, and also to support the healing process (Garden et al. 2007). Although anorexia is common following surgery, this is generally transient as recovery progresses and the effects

on nutritional status tend to be short-term unless complications, such as infections, occur. Tumours affecting the gastrointestinal tract are very rare in children (Stiller 2004, Kliegman 2007), but those affected can potentially be at an increased risk of undernutrition due to reduced intake and malabsorption of macronutrients and micronutrients, especially fat and, thereby, fat soluble vitamins. This has been reported in studies performed in adults (Bae et al. 1998). Studies investigating the acute and short-term effects of surgery on the nutritional status of paediatric cancer patients are very limited; with only one case report published reporting that an adolescent boy aged 15 years underwent gastrectomy due to a rare diagnosis of gastrointestinal adenocarcinoma. This patient presented very undernourished, required oral feeding and parenteral nutrition (PN) post-surgery and was discharged after 13 days. At four months follow-up, the patient was well-nourished (Slotta et al. 2011). Therefore, paediatric cancer patients undergoing surgery are likely to be at risk of undernutrition immediately after surgery. However, little is known about the long-term effects of gastrectomy and other surgeries performed in this population.

Finally, haematopoietic stem cell transplantation (HSCT) involves the transplant of haematopoietic stem cells, which derive mainly from matched bone marrow donors; however, in recent years there has been an increase in the use of umbilical cord blood cells (Veys & Rao 2004). HSCT is less commonly used than the previous forms of treatment due to the high mortality risk and numerous complications (Veys & Rao 2004). Hence, it is mainly restricted to high-risk ALL patients who do not respond to standard chemotherapy treatment, early AML relapse or in high-risk infant ALL treatment (Veys & Rao 2004). HSCT is preceded by an intensive chemotherapy regime aimed at both eradicating all malignant cells and providing immunosuppression to avoid rejection of the donor' stem cells. Thereby, the risk of undernutrition starts with chemotherapy induced toxicities, which is then exacerbated by the HSCT. Additionally, long term-effects including poor growth and reduced HFA have been reported in the literature (Leiper 2002b, Leiper 2002a).

1.3.2.5 Complementary and alternative medicine (CAM)

Despite the overall increase in survival rates as a result of the improvements in conventional cancer treatment already described, children still suffer major adverse effects, which may themselves not be relieved by conventional medicine (McLean & Kemper 2006). For this reason many families seek complementary medicine (Bishop et al. 2010). CAM has been defined as "a group of diverse medical health care systems, practices and products that are not presently considered to be part of conventional medicine" (NCCAM 2009). The most popular CAM worldwide are herbal remedies (Fernandez et al. 1998, Genc et al. 2009, Slayton 1997), diets and nutrition (Fernandez et al. 1998, Kelly et al. 2000, Leis 2000) and faith healing (Slayton 1998, Weyl Ben Arush et al. 2006, Yeh et al. 2000). In the UK however, the most popular therapies are; aromatherapy and massage (Cubbin 2004, McCann & Newell 2006), followed by high dose multivitamins, diets as well as music therapy (Cubbin 2004). In contrast, herbal remedies are not commonly used (Cubbin 2004, McCann & Newell 2006). CAM therapy has the potential to significantly improve the quality of life of childhood cancer patients and their nutritional status by reducing stress and alleviating symptoms (Kelly 2004, 2008, La 1994). However, many therapies, especially those from biological origin, can interact with conventional cancer treatment increasing their toxicity or reducing the efficacy of the actual treatment (Ladas et al. 2004) and in turn have detrimental effects on nutritional status.

Complementary therapy specialists providing CAM are now commonly employed in oncology units in the UK, often funded by charitable sources (Cancer Research UK 2012). A study performed in the paediatric oncology population from SE Scotland showed that 51% of patients used CAM and the most common therapies were multivitamins followed by massage therapy and fish oils (Revuelta-Iniesta et al. 2014), which is consistent with findings from other two studies performed in the UK (Cubbin 2004, McCann & Newell 2006) and a Finnish study, in which multivitamins, massage therapy along with aromatherapy were reported to be the most prevalent (Mottonen & Uhari 1997). Overall, 81% of paediatric cancer patients from SE Scotland perceived CAM to be beneficial during the course of treatment

(Revuelta-Iniesta et al. 2014); consistent with findings published somewhere else (Cubbin 2004, Ladas 2004).

However, at present there is an absence of evidence demonstrating the safety and efficacy of biologically based therapies. Consequently their use remains controversial owing to their potential interaction with conventional treatments (Cancer Research UK 2012). On the one hand it has been hypothesised that high doses of vitamins and minerals with anti-oxidant functions reduce the efficacy of cytotoxic therapies. This is because the latter function by generating free radicals and oxidative stress, which kill cancer cells; an effect which is counteracted by such antioxidants (Ladas et al. 2004). On the other hand individuals with depleted antioxidant status might benefit from this after treatment (Ladas et al. 2004). It has also been shown that other vitamins and minerals, for example selenium increases the toxicity of agents used in chemotherapy (Ciplastin). Meanwhile, high-dose vitamin E intake can interact with other treatment modalities as it has been associated with an increased in clotting time, which in turn can increase the risk of post-surgery bleeding (Kelly 2004).

Of all the biological agents, most adverse effects have been associated with the use of herbal remedies, including Echinacea (Kelly 2004), which has been reported to be used by 12% of the CAM users from SE Scotland (Revuelta-Iniesta et al. 2014). Echinacea might increase the effect of immunosuppressant drugs, such as corticosteroids or cyclosporine and the incidence of hepatotoxicity with agents such as Methotrexate (Kelly 2004, Post-White et al. 2006). Although 60% of families from this study reported a positive attitude towards the use of this herb (Revuelta-Iniesta et al. 2014), it was concluded that it is important to discuss potential interactions of CAM material with specialist pharmacists to inform families about the possible adverse effects and adopt a cautious attitude until more high-quality evidence is published. Indeed, due to the paucity of evidence high-quality interventional studies, which investigate the effects and possible interactions of biological based therapies as well as the efficacy and effectiveness of alternative health care therapies on nutritional status, are warranted.

Although the usage of CAM in paediatric cancer patients from SE Scotland has been investigated as part of the ENRICC project, this will not be presented and discussed in this thesis.

1.2.3 Social and psychological influences of the cancer and its associated treatment on the child and its family

Social and psychological factors play an important role in the aetiology and pathophysiology of malnutrition. In particular, studies have focused on learned food aversion, physical inactivity and behaviour issues due to the consequences of a long lasting treatment. Learned food aversion develops when the child associates specific foods with treatment induced side-effects, including nausea and vomiting, which often results in avoidance of these specific foods (Scalera 2002). In turn, this may limit choices (Bernstein 1978, Bernstein et al. 1982), which in this context can have detrimental consequences on long term food choices, affecting the nutritional status of childhood cancer survivors. Additionally, numerous reviews have been published reporting that habits, such as sedentary behaviour may develop during treatment, most possibly as a result of the length and intensity of the treatments (Reilly 2009a, Stolley et al. 2010, Winter et al. 2010, San Juan et al. 2011). These habits may persist, thus increasing the risk of obesity in later life (Reilly 2009a, Stolley et al. 2010). Finally, sequelae from the treatment may have long term consequences in behaviour. In particular, adopting both a permanent sick role and secondary gain have been described in patients and also survivors (Mertens et al. 2001), which may lead not only to nutritional issues, but also overall lifestyle problems.

In conclusion, it can be seen that there are many factors at interplay in the aetiology and pathophysiology of malnutrition in paediatric cancer patients with many gaps in the literature. In order to understand the mechanisms, it is first essential to elucidate the epidemiology of malnutrition in paediatric cancer patients, including the prevalence and specific patterns of change over time. Thereby, allowing to identify those children who might be at risk of becoming malnourished during and after the completion of treatment.

1.4 ANTIOXIDANTS, OXIDATIVE STRESS AND POLYUNSATURATED FATTY ACID STATUS IN PAEDIATRIC CANCER PATIENTS

The nutritional profile of paediatric cancer patients assessed by measuring plasma antioxidants, oxidative stress and polyunsaturated fatty acids (PUFA) has not been previously investigated in Scotland and there are very few comprehensive published studies performed elsewhere. In clinical practice, a nutritional profile tends to be subjectively assessed from a range of anthropometric measures and routine biochemical and haematological blood tests and non-routine plasma vitamin and mineral concentration. The antioxidant capacity of the blood, assessed from antioxidant status and markers of oxidative stress, provides a further indicator of both the nutrient adequacy as well as functional markers. Abnormal antioxidant and plasma fatty acid profiles have previously been described in children and adults diagnosed with and treated for cancer (de la Torre Aguilar et al. 2012, Kuliszkiewicz-Janus et al. 2008, Gleissman et al. 2011, Ladas et al. 2004). Although little is known about the potential effects of this in paediatric cancer patients, it is fair to assume that there may be functional implications (Guerra et al. 2007). In order to enhance the nutritional profile of paediatric feeds antioxidants such as carotenoids and long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA), primarily Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA), have been added. As results from phase-I of this project showed, almost half of the Scottish paediatric oncology patients required enteral nutrition support, mainly via nasogastric tube (NG-tube) (Paciarotti I 2013); however, at present there is no data to indicate any impact of these developments and their habitual dietary intake on their nutritional status and whether this has implications on clinical outcome.

1.4.1 Antioxidant and oxidative stress status of paediatric cancer patients

At present, there is a great deal of interest in the benefits of antioxidants on health. In particular, attention has focused on their protective role against cardiovascular diseases, neurodegenerative disorders and cancer (Ladas et al. 2004, Obrenovich et al. 2011). In order to understand the role of antioxidants and oxidative stress in paediatric cancer patients, it is first essential to describe oxidative stress, antioxidant

status and antioxidant capacity under normal circumstances and with a diagnosis of cancer.

Free radicals (FR) and reactive oxygen species (ROS) are essential for normal body functions (Conklin 2000). FR are involved in cell signalling and function as bactericidal in response to infection and, ROS are secreted as by-products in mitochondrial respiration and play an essential role in homeostasis (Conklin 2000). As previously discussed, cancer generates FR and ROS induced by an inflammatory response. Additionally, some forms of cancer treatment, including the use of alkylating agents, antimetabolites and radiotherapy generate FR to induce cancer-cell apoptosis (Ladas et al. 2004). FR are electrically charged molecules. As such, they capture electrons from other substances to neutralise themselves. Consequently, this leads to chain reactions, which in turn cause cell injury (Granot and Kohen 2004) by promoting lipid peroxidation, oxidative mediation of proteins and by damaging nuclear and mitochondrial DNA (Ou et al. 2001, Girard-Lalancette et al. 2009). Although FR are essential for life, excess production of FR and ROS not only damage cancer cells, but also cause injury to healthy cells and organs (Battisti et al. 2008).

Antioxidants are classified as endogenous and exogenous and function interactively and synergistically to neutralise FR. Endogenous antioxidants are produced by the human body and can either be enzymes, which catalyse FR quenching reactions or, metal binding proteins, which sequester free iron and copper ions that are capable of catalysing oxidative reactions. Exogenous antioxidants, such as vitamin C or selenium, are diet derived and function by neutralising FR and enhancing endogenous antioxidant activity (Ladas et al. 2004). Therefore, exogenous antioxidant status can be defined as static micronutrient markers, whilst antioxidant capacity is a measure of functional enzymes and micronutrient markers. Static markers refer to the concentration of vitamins and minerals in the blood, with potential antioxidant capacity, at a single point in time, which are then compared to reference data (Department of Health 1991). Such measures may not indicate whether nutrition metabolism is optimal or whether there are any functional

abnormalities (Clayton and Rounds 1994). It is functional markers that measure nutrients' activity in the body, such as the strength of the antioxidants against oxidation (Clayton and Rounds 1994).

Despite the potential protective role of antioxidants against cancer, there is discrepancy regarding their protective role during cancer therapy with few evidence published examining antioxidants status and their role during cancer treatment (Battisti et al. 2008, Mazor et al. 2008, Lamson and Brignall 1999). It has been suggested that the failure of dietary antioxidants to prevent further progression of cancer cells might be due to their inability to scavenge ROS at the site of production (Chandel and Tuveson 2014). Furthermore animal and *in-vitro* studies postulate that dietary antioxidants may stimulate oncogenic proteins, which otherwise would be attenuated by ROS, and inactivate some tumour suppressor proteins, such as P53, which are normally activated by ROS (Chandel & Tuveson 2014). Antioxidants may also promote the survival of detached cancer cells by preventing oxidative injuries to DNA (Saeidnia & Abdollahi 2013) and may reduce treatment efficacy (Salganik 2001, Labriola and Livingston 1999, Ray et al. 2000, Pathak et al. 2005).

On the other hand a systematic review (Ladas et al. 2004) and numerous narratives reviews have been published reporting that the effects of antioxidants are unlikely to be harmful; however, their role regarding their benefits on clinical outcomes is still in dispute (Ladas et al. 2004, Block 2004, Prasad 2004, Prasad et al. 1999, Prasad 2005, Prasad 2003, Prasad et al. 2003). Two mechanisms by which antioxidants may be of benefit against cancer cells have been proposed: (i) the direct effect of antioxidants on tumour cells and (ii) the effects of antioxidants in combination with chemotherapy and radiotherapy. (i) *In vitro* and animal studies have shown that some antioxidants induce cell differentiation and inhibit the growth of several tumour cells, thus reducing the growth of certain types of tumour cells (Labriola and Livingston 1999, Prasad et al. 1999, Agus et al. 1999); (ii) *in vitro* and very few *in vivo* studies have proposed that some antioxidants, including vitamin A (and β-carotene) (Teicher et al. 1994, Rutz and Little 1989), vitamin C (Prasad 1980) and vitamin E (Chinery et al. 1997, Prasad et al. 1980), used with standard cancer therapy may enhance the

growth inhibitory effect of chemotherapy agents on some cancer cells and, also protect normal body cells from toxicity (Prasad et al. 1999, Kumar et al. 2002, Prasad et al. 1994).

In order to learn about antioxidants and oxidative stress in paediatric cancer patients at diagnosis and during therapy, and whether they have any protective or detrimental influence on clinical outcome, it is vital to review current evidence on this topic. Thus, an electronic search of the English language was performed (no restriction-April 2014) using the Cochrane Library, MEDLINE (via EBSCOhost), CINHAL (via EBSCOhost) and PUBMED to identify systematic reviews, Randomised Control Trials (RCT) and observational studies. The reference list of all relevant articles was also examined. The initial search strategy identified the following keywords: "paediatrics", "cancer", "cancer treatment", "antioxidant status", "antioxidant capacity" and "oxidative stress" and, adaptations for British and American English were made for all searches. The eligibility criteria included studies investigating children and young people aged less than 18 years, diagnosed with and treated for cancer (diagnosis according to ICCC-3 criteria) and also investigating total antioxidant status (TAS), total antioxidant capacity (TAC) and/or oxidative stress in paediatric cancer patients.

The primary search yielded 388 studies and, of these, only 23 met the eligibility criteria. A summary of these studies is presented in table 1.3. Four studies were excluded because they were performed in adults instead of children (Brackett et al. 2012, Calautti et al. 1980, Drozda et al. 2010, Jonas et al. 2000). Most of the studies investigated antioxidant status and/or antioxidant capacity (n=19) and oxidative stress (n=14). But very few assessed whether antioxidant status, antioxidant capacity or oxidative stress was either predictive of clinical outcome or had any effect on clinical outcome (n=8). Additionally, all studies were either prospective cohort or cross-sectional studies and a control was used in the majority as reference values.

1.4.1.1 Antioxidant status and antioxidant capacity in paediatric cancer patients

Evidence investigating TAS and TAC in paediatric cancer patients at different stages of the disease, especially after the completion of therapy, is limited as only 19 studies have been published to date (see table 4.1). Despite this, most studies apart from two (Papageorgiou et al. 2005, Pazirandeh et al. 1999) are in agreement with the fact that both antioxidant status and antioxidant capacity are lowered in patients diagnosed with cancer in comparison to control groups at the time of diagnosis (Battisti et al. 2008, Al-Tonbary et al. 2011, Kennedy et al. 2004a, Krawczuk-Rybak et al. 2012, Malvy et al. 1997, Malvy et al. 1997, Misaki et al. 2003, Neyestani et al. 2007, Nathan et al. 2011, Sentürker et al. 1997, Fiore et al. 1997) and as expected, TAS and TAC are reduced further during the course of treatment (Mazor et al. 2008, Neyestani et al. 2007, Papageorgiou et al. 2005, Pazirandeh et al. 1999, Pazirandeh et al. 1999, Protas et al. 2010). Only two studies have investigated TAS and TAC after the completion of therapy and they both reported no difference between survivors of childhood cancer and the control group (Krawczuk-Rybak et al. 2012, Papageorgiou et al. 2005).

A number of limitations have been common to most studies: (i) it can be seen from the table that most studies investigated children diagnosed with ALL and very few focused on solid and brain tumours, (ii) the type of dietary and endogenous antioxidants measured and the techniques employed to assess antioxidant capacity were very variable, making once more the comparison between the different articles very difficult. Of note, all techniques used in the studies were validated. (iii) Dietary intake, the use of nutritional support and vitamin and mineral supplementation was a major confounding factor in most studies. (iv) None of the studies assessed treatment induced side-effects such as malabsorption. Despite these limitations exogenous antioxidant status and its antioxidant capacity as well as endogenous antioxidants will be described.

- **Antioxidant status**

(i) Vitamins A, E and C

Changes in plasma vitamin A, E and C in children diagnosed and treated for cancer have been described in very few studies. Three studies were found in the literature to investigate Vitamin A (Malvy et al. 1997, Fiore et al. 1997, Kennedy et al. 2004b, Kennedy et al. 2005). Of these, two assessed changes in plasma vitamin A in children diagnosed with any type of malignancy (Malvy et al. 1997, Fiore et al. 1997), whilst one study examined plasma vitamin A in children diagnosed with ALL only (Kennedy et al. 2004b). Malvy (1997) and Fiore and co-workers (1997) reported significantly lower levels of vitamin A (and β-carotene) in children diagnosed with cancer at the time of diagnosis in comparison with matched healthy controls. Plasma vitamin A remained low following six months of treatment (Malvy et al. 1997). Only, Fiore et al (1997) assessed dietary intake using a 48-hours dietary recall and found that 33% of the cohort consumed less than 80% of the recommended daily allowances. The authors proposed that the low level of vitamin A seen in this population was induced by the cancer itself instead of the poor dietary intake (Fiore et al. 1997). Although, this might be the case, it can be argued that a 48-hours dietary recall is not a very accurate measurement of assessing dietary intake, especially micronutrients, as it tends to underestimate both macronutrients and micronutrients (Burrows et al. 2010). Additionally, the authors reported total energy intake but vitamin A intake was reported in neither the cohort nor the controls. Finally, the cut-off value established by the authors (less than 80% of the recommended daily allowances for energy intake) might have underestimated their findings.

In contrast, Kennedy et al. (2004) found that children diagnosed with ALL (n=103) had both poor vitamin A intake and levels at diagnosis. This increased during treatment (at six months) despite vitamin A intake remaining below recommendations. There was no correlation between vitamin A intake and plasma vitamin A, which was attributed to the methods used to assess dietary intake: 24-hour dietary recall and the food frequency questionnaire. It has also been discussed that an

increase in plasma vitamin A in ALL patients occurs as a result of treatment with prednisone (Kennedy et al. 2005). In normal circumstances, 50-80% of vitamin A is stored in the liver and this is used in periods of deficiency while maintaining normal plasma vitamin A (Yu et al. 1994). For vitamin A to be transferred to target tissues it first needs to be de-esterified and bound to retinol binding protein (RBP). Prednisone is thought induce the synthesis of RBP, thus causing an increase in plasma vitamin A (Yu et al. 1994)

Plasma vitamin E and α-tocopherol (organic chemical compound with vitamin E activity) in paediatric cancer patients has been studied by four groups (Battisti et al. 2008, Kennedy et al. 2004a, Malvy et al. 1997, Misaki et al. 2003). Of these, three studies included children diagnosed with ALL only (Battisti et al. 2008, Kennedy et al. 2004a, Misaki et al. 2003), whilst one examined erythrocytes α-tocopherol and lymphocytes α-tocopherol concentration in children diagnosed with a malignancy included in the ICCC-3 (Malvy et al. 1997). It appears that children diagnosed with ALL tend to have normal vitamin E levels at the time of onset (Kennedy et al. 2004a, Misaki et al. 2003); however levels decrease with treatment (Battisti et al. 2008, Kennedy et al. 2004a, Misaki et al. 2003) and return to normal thereafter (Battisti et al. 2008). Similar findings have been reported in adults diagnosed and treated for chronic myeloid leukaemia (Ghalaut et al. 1999, Singh et al. 2000). Only, Battisti et al. 2008 reported low vitamin E at the time of onset, which was attributed to an increased in oxidative stress. Finally, Malvy et al. (1997) reported an increased risk of vitamin E (α-tocopherol) deficiency at diagnosis, which only continues throughout treatment in children diagnosed with bone tumours. Of note, neither Battisti et al. (2008) nor Malvy et al. (1997) measured dietary intake, which could have also been the reason for the low vitamin E levels as it has been reported by Kennedy et al. (2004), who found that 85% of children had a vitamin E intake below recommendations. Moreover, none of the above studies described treatment induced side-effects, such as vomiting or mucositis, which would reduce absorption of nutrients.

Alterations in plasma vitamin C show more consistent results. The three studies found in the literature investigated vitamin C in children diagnosed with ALL at

diagnosis (Kennedy et al. 2004a) and during treatment (Kennedy et al. 2004a, Neyestani et al. 2007, Kakar et al. 1975). Although plasma vitamin C was reported to be within the normal range at diagnosis (Kennedy et al 2004a), this was significantly lower in the cohort than in the controls during treatment, despite vitamin C intakes being higher in the cohort group (Kennedy et al. 2004a, Neyestani et al 2007). These findings suggest that there might be an increased use of vitamin C due to cancer treatment in patients treated for ALL, which in turn may increase vitamin C requirements.

(ii) Zinc, Copper and Selenium

Zinc, copper and selenium have antioxidant properties. In healthy (non-stressed) individuals the plasma concentration of these micronutrients is a reliable indicator of the body's status. However, they are also acute phase reactants and their plasma concentration may change during inflammation; zinc and selenium concentration may decrease, whilst copper levels may increase (Galloway et al. 2000). Despite this, two studies have investigated the changes in zinc and copper as a measured of antioxidant status in children diagnosed with ALL (Sgarbieri et al. 2006) and a malignancy according to the ICCC-3 (Malvy et al. 1997). These patients were also treated with chemotherapy at the time of the studies. Their findings are contradictory (see table 3.1). Furthermore, two studies investigated plasma selenium in this population (Malvy et al. 1997, Pazirandeh et al. 1999). Once more, findings are inconsistent. Pazirandeh and co-workers (1999) investigated plasma selenium in children diagnosed with ALL and AML and compared this with a matched healthy control. At diagnosis, the authors found no differences between the ALL and the control group; however, the AML group had significantly lower selenium levels than the control group. Plasma selenium did not change in the AML group after induction chemotherapy, whilst this was significantly reduced in the ALL group. In contrast, Malvy and co-workers (1997) found no difference in plasma selenium when the cancer (ICCC) group was compared to the control group at the time of diagnosis. However, the cancer group had significantly higher selenium levels after 6 months of treatment in compare to healthy controls. As none of the studies reported markers of

inflammation, treatment induced side-effects; especially malabsorption, kidney and liver impairment, and only one study (Sgarbieri et al. 2006) reported dietary intake, the causes of these changes are still unknown. Additionally, the inconsistent findings could also be attributed to the differences in the cancer diagnosis and the different treatment regimes.

- **Total antioxidant capacity**

Total antioxidant capacity (TAC) has been assessed by measuring the endogenous antioxidants enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) and or by measuring the scavenging strength of exogenous antioxidants (see table 1.3). All studies, apart two (Nathan et al. 2011, Stachowicz-Stencel et al. 2011) included children diagnosed with ALL (Battisti et al. 2008, Mazor et al. 2008, Al-Tonbary et al. 2011, Neyestani et al. 2007, Sentürker et al. 1997, Papageorgiou et al. 2005, Protas et al. 2010, Nakagawa 2000). As expected most studies agreed that TAC was lowered in children with cancer in compared to controls and that this was reduced further during cancer therapy. Additionally, those children treated with high risk protocols (ALL) or considered to have a poorer prognosis at diagnosis had lower TAC than those treated with standard risk protocols (ALL) or considered to have a good prognosis (Al-Tonbary et al. 2011, Stachowicz-Stencel et al. 2011). Interestingly, only one study investigated changes in TAC throughout all the stages of disease (diagnosis, treatment and after therapy) and compared it against a control group (Papageorgiou et al. 2005). The authors reported that TAC in children diagnosed with ALL, AML or a CNS tumour was not significantly lowered than that of the cancer-free controls either at diagnosis or after the completion of therapy. Notably, the study did not mention whether the controls were healthy at the time of the study, only that they were cancer free.

1.4.1.2 Oxidative stress

Although the number of studies assessing oxidative stress in this population is scarce, all studies reported consistent findings. Oxidative stress increases at diagnosis and during the course of therapy (or it is higher than healthy controls) and, this is

regardless of the type of diagnosis and the technique employed to assess it. These findings are consistent regardless of the technique used to assess oxidative stress: by lipid peroxidation (Battisti et al. 2008, Al-Tonbary et al. 2011), DNA damage (Sentürker et al. 1997), oxidation of the cerebrospinal fluid (CSF) (Protas et al. 2010, Caron et al. 2009, Miketova et al. 2005, Stenzel et al. 2010) or protein carbonylation (Battisti et al. 2008, Mazor et al. 2008, Stachowicz-Stencel et al. 2011).

As it has already been discussed oxidative stress is part of the normal body functions and it is induced by the cancer treatment in order to kill cancer cells; therefore, these findings are reassuring. The questions are whether oxidative stress causes irreversible damage to the body, which can be detected after treatment, whether the degree of oxidative stress at diagnosis and during therapy can predict clinical outcome and whether tissue damage can be avoided or reduced with antioxidant intake. Although, there is a lot of evidence reporting long term side-effects of childhood cancer survivors (Wallace et al. 2013), only one study has addressed the first question (Battisti et al. 2008). The authors found that lipid peroxidation and protein carbonylation not only was high during treatment but also remained high after the completion of therapy, suggesting irreversible damage to tissues. The second question will be discussed in the next section and the third question has been discussed previously (1.4.1.1 Antioxidant status).

Table 1-3 Studies measuring antioxidant status, antioxidant capacity oxidative stress

Author	Quality	Patients Dx	N, age years	Time of measurement and method	Method		Results		
					Design	Parameters	At diagnosis	During treatment	After treatment
Al-Tonbary et al. (2011) Egypt	Weak	ALL Controls (outpatient clinic of general paediatric surgery)	ALL: N=50 Age: mean±SD (range): 6.84±3.73 (1.5-12) y Controls: N=10 (matched for age and sex)	T: At diagnosis and after 5 weeksof treatment (end of induction therapy) M: Measurement of serum TAC: Koracevic et al 2001 Measurement of MDA (lipid peroxidation): Satoh K 1978 and Ohkawa et al 1979 Apoptosis DeadEnd™ Fluorometric TUNEL System (Promega Corp., Madison, WI, USA)	PCS	TAC, MDA, apoptosis	Controls had higher TAC (*p<0.0001*) and lower MDA (*p<0.0001*) than ALL cases ALL cases: No sig difference between standard and high risk groups for MDA, TAC and apoptosis	ALL cases: MDA sig ↑ from diagnosis (*p<0.001*) TAC sig ↓from diagnosis (*p=NR*) Apoptosis sig ↑from diagnosis (*p<0.001)* TAC was sig lower in the high risk group than the standard risk group (*p=0.024*), but no difference was found in MDA between the groups	
Battisti et al. (2008) Brazil	Weak	ALL Controls (healthy	ALL: N=80 Age range: Just	T: JD, RI, RM, OT M:	C	Oxidative stress: lipid and protein oxidation	Lipid peroxidation was ↑ in the JD, RI in	NA	NA

	volunteers, age and SES matched)	diagnosed (JD): 3-17 y Remission induction (RI): 3-19 y Remission maintenance (RM): 3-23 y Out of treatment (OT): 4-20 y Controls: N=50 (matched for age and SES)	TBARS (lipid peroxidation): Jentzsch et al1996 CAT activity: Nelson and Kiesow 1972 SOD activity Mc Cord and Fridovich 1969 Non-protein thiols: Ellman GL 1959 Serum vitamin E: Hansen and Warwick 1969 Protein carbonylation (protein oxidation): Bradford MM 1976	Endogenous antioxidants (CAT, SOD) Exogenous antioxidants (vitamin E)	compare to controls, RM and OT (*p<0.001*) CAT activity was ↓ in JD, RI, RM compared to controls (*p<0.001*) SOD activity ↓ JD, RI compared to controls (*p<0.01*). Controls, RM and OT (*p>0.05*) Non-protein thiols levels were ↓ at JD, RI and RM in compare to controls (*p<0.001*) Vitamin E was ↓ JD and RI (*p<0.001*) Nodifference between

							controls, RM and OT patients	
Caron et al. (2009) USA	Moderate	ALL	N=88 Mean(±SD) 7.1(±3.1)y	T: from diagnosis to 3 years Diagnosis, post-induction, post-consolidation and end of therapy M: Oxidised changes in CSF PC: Miketova et al 2005 Executive function measures: The Behavior Rating Inventory of Executive Function (BRIEF): Gioia et al 2000 higher scores reflect more pathology	PCS	Oxidised changes in CSF		Oxidised PC ↑ from diagnosis to post-induction (*p<0.001*) and post-consolidation (*p<0.001*)
Fiore et al. (1997) Italy	Moderate	Newly diagnosed malignancy Healthy controls	Cases n=54 mean(range) 5.6(0.7-16.5)y Healthy children mean n=47 mean(range) 5.9(0.7-15.5)y	T: at diagnosis M: Vitamin A measured by high performance liquid chromatography (pump model 200LC, Perkin-Elmer) Reference values: age and sex-specific Italian recommended daily	PC	Plasma Vitamin A Dietary vitamin A and total energy intake	33% (18) consumed less than RDA 26%(14) had extremely low levels of Vitamin A Vitamin A levels were significantly	

				allowances Cases' vitamin A levels were compared against healthy controls			lower in the cases compared to the control group ($p<0.001$; 95% CI: 288-412)	
Kakar et al. (1975) Ireland	Weak	ALL Controls (cancer-free): congenital hip dislocation, congenital talipes, tonsils and adenoids and fracture of femur	Cases n=10 Age range 4-11 y Controls n=10 Age range 4-14 y	T: during treatment M: Plasma ascorbic acid concentration measured using dinitrophenyl-hydrazine (Loh and Wilson 1971)	CS	Plasma and leukocytes ascorbic acid		Ascorbic acid from plasma ($p<0.001$) and leukocytes ($p<0.05$) were sig lower in ALL than in controls
Kennedy et al. (2004) USA	Strong	ALL	N= 103 Age mean (±SD)y Standard-risk protocol 4.7(±2.6)y High risk protocol 10.5(±4.6)y	T: at diagnosis, 3-4 months and 6-7 months post diagnosis M: Total ascorbate analysis: HPLC analysis (Behrens et al 1987) Vitamin A and E: HPLC analysis (Bieri et al 1979) Total carotenoids Colorimetric procedure (Roels et al 1972)	PCS	Total ascorbate Vitamins A and E Total carotenoids HDL and LDL Ch and TG	Percentage of children with TEI below recommendations (FFQ/24-dietary recall) Vitamin A 49/54% Total carotenoid 33% B-carotenoid 88% Vitamin C 10/13% Vitamin E 83/85%	Plasma vitamin A : ↑ from diagnosis at 3 (*$p<0.001$*) and 4 months (*$p<0.001$*) Plasma total carotenoids expressed per TCh No change Plasma vitamin C: ↑ at 3 months

				Dietary intake: 24h recall and FFQ Dietary analysis: Minnesota NUTRITION DATA SYSTEM (NDS) software Recommended dietary allowance (National Health and Nutrition Examination Survey 2000) Plasma antioxidant cut-off values NR		Normal ranges for plasma antioxidants NR	($p=0.04$) and ↓ at 6 months ($p=0.007$) Plasma vitamin E/Ch: ↓at 3 months and 6 months ($p=0.02$) Plasma vitamin E: ↓at 3 months ($p=0.005$) and 6 months (p=0.02)
Kennedy et al. (2005) USA	Strong	ALL	N= 103 Age mean (±SD)y Standard-risk protocol 4.7(±2.6)y High risk protocol 10.5(±4.6)y	T: at diagnosis, 3-4 months and 6-7 months post diagnosis M: Total ascorbate analysis: HPLC analysis (Behrens et al 1987) Vitamin A and E: HPLC analysis (Bieri et al 1979) Total carotenoids Colorimetric procedure (Roels et al 1972) Antioxidant capacity (ORAC): Cao G and	PCS	Exogenous antioxidants (vitamins A and E, carotenoids) Antioxidant capacity Oxidative stress		↓ ORAC ↑8-oxo-dG

				Prior R 1995 Oxidative damage (8-oxo-dG): Yarborough et al 1996				
Krawczuk-Rybak et al. (2012) Poland	Weak	Survivors: ALL, AML, NHL, HD and solid tumours Healthy controls Newly diagnosed ALL	Survivors: N=100 Age median(IQR) 12.7(8.68-16.55)y Healthy controls N=22 Age mean(±SD): 12.6(±4.79)y Newly diagnosed ALL: NR	T: Survivors: mean(±SD) since cessation of treatment 5.3(±2.99)y Newly diagnosed ALL: At diagnosis During therapy M: TAS: RANDOX TAs kit (Randox, Ardmore, U K) Measurements of the Metabolic syndrome (MS): Weight, height and blood pressure: conventional techniques Abdominal obesity: Waist to hip ratio Biochemical test: automated directed methods	CC	Plasma TAS	TAS was ↓ in newly diagnosed ALL compared to survivors an d healthy controls	TAS did not differ between survivors and healthy controls
Mazor et al. (2008) Israel	Weak	ALL Solid tumours Controls	N= 13 ALL (n=7) Age mean (±SD) NR	T: During treatment M: Oxidative stress:	CS	Antioxidant capacity: Thiols, FRAP, TEAC and	Children with ALL: Thiols ($p<0.01$) and FRAP	

		(healthy adult controls)	Solid tumours (n=6) Age mean (±SD) 12.5(±5.6)y Healthy adult controls NR	plasma total thiols: DTNB reduction method (Miao-Lin H 1994) Radical scavenging activity: DPPH method (Brand-Williams et al 1995) Antioxidant capacity: FRAP method (Benzie and Strain 1996) and TEAC method (Miller et al 1993)		DPPH		(*p<0.01*) were ↓compared to healthy adult controls Solid tumours: thiols, FRAP and TEAC were normal. DPPH was ↓ compared to controls (*p<0.01*)
Miketova et al. (2004) USA	Moderate	ALL	N=21 Mean (±SD) 6.26(±2.1) Low-risk n=7 Standard-risk n=7 High risk n=7	T: diagnosis, induction, consolidation and continuation treatment M: PC oxidation from the CSF (folch and others 1957)	PCS	CSF phospholipid oxidation	At diagnosis; non-sig difference between the 3 groups	PC oxidation: Almost no ↑ in the low-risk group Standard risk 2-fold ↑ High-risk 3-fold ↑
Malvy et al. (1997) France	Weak	ICCC Controls (healthy children)	Cases n=170 Age range (0-16) y Mean/median age NR Controls n= 632 Age range (0-16) y	T: at diagnosis and 6 months M: For retinol, alpha-tocopherol and beta-carotene: high performance liquid chromatography assay (Malvy et al 1993) Zinc and Selenium:	PCS	Micronutrients with antioxidant function: retinol, alpha-tocopherol, beta-carotene, zinc and selenium	Mean serum concentrations of retinol, beta-carotene, alpha-tocopherol and Zinc were sig lower in cancer patients compared to	Retinol ↑ sig during treatment Zinc ↓ sig during treatment No other changes. Leukaemic

			Mean/median age NR	Atomic absorption technique by Neve et al 1987 Reference values: healthy French children (control)			controls. No sig difference in Se	tended to have higher retinol and Se levels after 6 months of treatment Lymphoma patients tended to have increased retinol levels Brain tumours: lower Zn Bone tumours: ↓α-tocopherol
Misaki et al. (2003) Japan	Weak	ALL Controls (healthy children, acute infections and epilepsy)	Cases Onset n=22 Mean(±SD) 5.7(±4.6) Remission n= 18 Mean (±SD) 6.4(±4.5)y Controls n= 22 Mean (±SD) 4.9(±4.0)y	T: at diagnosis and remission M: Plasma α-tocopherol: Tamai et al 1988 Lipid peroxidation: 8-isoprostane: Enzyme immunoassay (EIA) Cayman Chemical, Ann Arbor, MI) Hoffman et al 1996 Acrolein: EIA Hoffman et al 1996	CC	Antioxidants: α-tocopherol (from erythrocytes, lymphocytes) Lipid peroxidation: 8-isoprostane and acrolein α-tocopherol gene expression	Erythrocytes alpha-tocopherol did not differ between the three groups Leukocytes α-tocopherol from untreated ALL children was sig lower than those in remission and controls Lipid	

						peroxidation did not differ between untreated patients and controls No sig difference was found in the alpha-tocopherol gene expression between the untreated and control groups.		
Nakagawa K. (2000) Japan	Weak	ALL Healthy controls	ALL on treatment n= 57 Mean (±SD) 9.7(±4.7)y ALL after treatment n=16 Mean(±SD) 8.7(±3.8)y Healthy controls n=31 Mean(±SD) 48(±14.7)y	T: undergoing chemotherapy treatment and after treatment M: serum and CSF ASA and ASR concentrations were measured using a stable free-radical TEMPOL (Wisconsin, USA) EPR	CC	ASA and ASR from CFS and serum		ASA and ASR concentrations were higher in CSF than serum ASA and ASR in serum and CSF good and moderate correlation (r=0.45; p=0.001 and r and p=NR): indicating that oxidation might be induced by	ASA and ASR in serum and CSF in patients after treatment were similar to those receiving treatment.

								treatment.
Neyestani et al. (2007) Iran	Moderate	ALL Healthy controls	ALL n= 28 Range (2-18)y Healthy controls n=30 Range (2-18)y	T: undergoing treatment M: blood TAC measured using Miller et al 1993 method and serum and urinary vitamin C described in the article. Reference NR	CS	Blood TAC Blood and urinary vitamin C Dietary intake		TAC and vitamin C (serum and urine) were 10-fold and 2.5-fold higher in controls than in patients despite dietary intake being higher in patients Serum TAC was lower in patients than controls
Nathan et al. (2011) Malaysia	Weak	Bone and soft tissue sarcoma Healthy controls	Cases N= 90 Bone tissue sarcoma n=27 Soft tissue sarcoma n=20 Controls n=40 Age range (7-78) y	T: diagnosis M: Lipid peroxidation: Plasma and urine; MDA TBARS Protein carbonyl: DNPH (Cayman's kits) Antioxidant status: Enzyme antioxidants: Plasma CAT activity (Cayman's catalase activity assay kit) PlasmaSOD activity; Cayman's SOD activity Assay Kit Non-enzymatic antioxidants Total Thiols; DTNB	C	Plasma lipid peroxidation, protein carbonyl, antioxidant status (CAT, SOD, TEAC)	Urine and plasma MDA sig higher in sarcoma patients compared to controls ($p=0.0001$) TEAC ($p=0.0001$) and total thiol ($p<0.05$) was sig lower in sarcoma patients compared to controls	

				(CALBIOChem, USA) TEAC: formation of ABTS radical (Sigma-Aldrich Inc., USA)			SOD and CAT were sig lower in sarcoma compared patients to controls (p=0.0001) Antioxidants and lipid and protein peroxidation: not difference was seen between the two types of sarcoma.		
Papageorgiou et al. (2004) Greece	Weak	Malignant diseases (ALL, CNS, AML) Control cancer free children	Cases n=20 mean(±SD)5.9±0.5)y Control cancer free children n=80 mean(±SD) 6.2(±0.9) *Is the control healthy?*	T: at diagnosis, during chemotherapy and 1 and 6 months after completion of therapy M: TAC and c-TAC TAC: TAC kit from Medicon SA (Gerakas, Greece) TAC and cTAC was calculated according to Kampa et al. 2003 ROS: cytometry according to Rothe et al. 1994	PCS	TAC and cTAC ROS	No sig difference were observed between cases and controls	Sig ↓ of TAC (*p=0.0007)* and cTAC (*p=0.002)*	No sig difference were observed between TAC and cTAC at diagnosis and at end of therapy
Pazirande	Weak	ALL	ALL n=40	T: before CT and at	PC	Se	No sig	Se sig ↓ during	

h et al. (1999) Iran		AML Healthy matched controls	AML n= 20 Age range 3-8y Controls Age range 3-8y	day 28 (during CT) M: Neutron Activation Analysis (reference: NR)			difference between the ALL and the control group AML had sig ↓ plasma Se than the control group	treatment in children with ALL only ALL group: Se levels pre-CT 110.7(±28.3)µg/L Se levels post CT 80.8(±15.5)µg/L ($p<0.001$)
Protas et al. (2010) Poland	Weak	ALL Controls (children diagnosed with cerebrospinal meningitis)	Cases n= 38 mean(range) 7.6 (2-16) y Controls n=22 mean(range) 8.23 (3-17) y	T: at diagnosis, after induction CT, during consolidation and before maintenance therapy M: TAC measured using Antioxidant capacity assay ImAnOX (Immundiagnostic) Oxidative stressed (8-Isoprostane) measured using 8-Isoprostane ImmunoAssay Kit (Cayman)	PC	TAC of CSF Oxidative stressed of CSF	CNS 8-isoprostane level mean±SD) 9.05(±1.6)pg/mL CNS 8-isoprostane did not sig correlate with leukocytes, organomegaly and dehydrogenase level TAC level mean(±SD) 203.1(±6.2)µmol/L	CNS 8-isoprostane level sig ↑ at every stage: After induction: mean(±SD): 24.8(±7.6)pg/mL (*$p<0.01$*) Consolidation: mean(±SD) 17.3(±2.2)pg/mL (*$p<0.05$*) Before maintenace: mean(±SD) 32.3(±7.9)pg/mL *($p<0.01$)* TAC level sig ↓ after induction: mean(±SD)

								189.8(±6.2) µmol/L During consolidation Mean(±SD) 188.3(±1.9) µmol/L
Senturker et al. (1997) Turkey	Weak	ALL Controls (disease-free children)	Cases n=10 Age range 3-12 y Controls n=10 Age range 3-12 y	T: at diagnosis (before treatment) M: blood samples CAT activity SOD activity GPx activity according to Beutler 1973, Paglia et al. 1967, Winterbourn et al. 1975. DNA damage measured with stable-labeled analogues and chromatography/mass spectrometry (Dizdaroglu 1994)	CC	CAT, SOD, and GPx activity in lymphocytes of children with ALL DNA damage	The levels of all three enzymes from lymphocytes in ALL patients were found to be sig lower than those of the disease-free children group. The mean values of the modified DNA bases was sig higher in ALL than controls (*p<0.05)*	
Sgarbieri et al. (2006)	Weak	ALL	N= 45 Median (range) age 5(1-11) y	T: Diagnosis, induction, reinduction, and maintenance therapy M: Zn and Cu	PC	Plasma Zn and Cu concentration Dietary Zn and Cu	Zn (±SD) 100.8(±25)µg/dl (within normal range) Cu	Zn levels did not change during treatment Cu levels ↓ with the induction

				measured using atomic absorption spectrophotometry			206(±75)µg/dl sig higher than normal range (*p<0.05*) Zn and Cu intake was 24% and 2%below DRI respectively	CT and remained stable during the rest of the treatment Dietary Zn and Cu ↑ during induction and re-induction therapy.
Stachowicz-Stencel et al. (2011) Poland	Moderate	STS NB Healthy controls	N=99 STS n= 47 median(range) 10.3(1.5-21.4) y NB n=52 median(range) 0.9 (0.2 - 17.8) y Healthy controls n= 30 median(range) 8.05(1.4-17.9) y	T: before CT and at the time of response assessment M: SOD in erythrocytes assessed according to Ransod (Randox, Crumlin, UK) procedure GPx assay in erythrocytes assessed according to Ransel (Randox) procedure Ischaemia modified albumin (IMA) assessed by calorimetric assay using dithiothreitol (DTT). Patients stratified	PC	Antioxidant enzymes: SOD and GPx Inflammatory marker: IMA	There was not statistically sig difference in the SOD and GPx activity between cases (STS and NB) and controls IMA was sig higher in the cases than in the control group	SOD and GSH-Px was sig lower in patients who had a poor response than to those who had a good response to treatment (*p=0.04*) SOD and GPx did not statistically differ between healthy controls and cases who had a good response IMA was sig higher in cases in compared to controls (*p=0.002*)

				according to: Good response and poor response			
Stenzel et al. (2010) USA	Strong	ALL	N= 87 mean(±SD) 7.0(±3.1) y Range 3-16 y	T: at diagnosis, post-induction , post-consolidation and end of therapy M: CSF Sample phospholipids measured according to Mawatari et al. 1998 Behavioral measures: Behavior Assessment System for Children (BASC) Reynolds et al. 1992	PC	CSF phospholipids (oxidation) Sex, age at diagnosis, treatment, SES, IV-MTX dose	At all 3 points age-adjusted mean T-scores for most of the individual BASC scales fell within the average range

ALL: acute lymphoblastic leukaemia;ASA: Ascorbate; ASR: Ascorbyl Radical; C: cohort study; CAT: Catalase; CC: Case control; Ch: Cholesterol; CNS: Central Nervous System;CS: cross-sectional study; CSF: Cerebrospinal fluid; CT: chemotherapy; c-TAC: Corrected Total Antioxidant Capacity; Cu: Copper; Dx: Diagnosis; EDI: Estimated Dietary Intake; EPR: Electron Paramagnetic Resonance; FFQ: Food Frequency Questionnaire; FRAP: Ferric Reducing Ability of Plasma; GPx: Glutathione Peroxidase; HD: Hodgkin's Disease; HDL: High-density Lipoprotein; ICCC-3: International classification of Childhood Cancer, Third edition; LDL: Low-density Lipoprotein; M: method; MDA: Malondialdehyde; MTX: Methotrexade; NA: non-applicable (i.e. not part of study aims); NB: Neuroblastoma; NHL: non-Hodgkin's lymphoma; NR: not reported and/or not clear; ORAC: Oxygen Radical Absorbance Capacity; PCS: prospective cohort study; RDA: Recommended Daily Allowance; SD: standard deviation; Se: Selenium; SES: Socio-economic status; sig: Singificant; SOD: Superoxide Dismutase; STS: Soft Tissue Sarcoma; T: time of measurement; TAC: Total Antioxidant Capacity; TBARS: Thiobarbituric Acid Reactive Substances; TCh: Total Cholesterol; TEAC: Trolox Equivalent Antioxidant Capacity; TG: Triglycerides; UK: United Kingdom; USA: United States of America; y: years; Zn: Zinc; ↓: decrease; ↑: increase.

1.4.1.3 Impact of antioxidants and oxidative stress on clinical outcomes

Table 1.4 describes the results of 8 studies investigating the impact of antioxidant status, TAC and oxidative stress on clinical outcomes. Although, most published studies agreed that high oxidative stress was associated with poorer outcomes, they were very heterogeneous in the outcomes measured (Al-Tonbary et al. 2011, Caron et al. 2009, Stenzel et al. 2010). Oxidative stress was associated with increased apoptosis (Al-Tonbary et al. 2011), poorer working memory and organization and, reduced attention at the end of therapy (Caron et al. 2009). Additionally, Stenzel and co-workers (2010) found that higher oxidation was significantly associated with increased behavioral problems at the time of treatment and at the end of therapy. These studies were all performed in children diagnosed and treated for ALL. Although, no studies have investigated oxidative stress in children diagnosed with other types of cancer, it is well established that the more serious the injury is the greater the stress response will be regardless of the diagnosis (Berger 2005).

Evidence investigating TAS and TAC and their impact on clinical outcomes appeared inconclusive. Two studies found that higher TAS, whether this is intake or plasma concentrations, was associated with the following: reduced chemotherapy induced side-effects, toxicity, delays in treatment and days of hospital stay as well as higher quality of life scores in children diagnosed with ALL (Kennedy et al. 2004a, Kennedy et al. 2005). These findings are consistent with studies performed in adults who are also diagnosed and treated for cancer, as reported in a systematic review (Ladas et al. 2004). Whilst, no correlation was found between zinc levels at any stage of the disease and treatment and prognosis of disease in children diagnosed with ALL (Sgarbieri et al. 2006). Similarly, two studies which investigated TAC, calculated by measuring the antioxidant enzymes SOD and GPx, found opposing results. Reduced TAC was associated with poorer response to therapy in children diagnosed and treated for soft tissue sarcoma and neuroblastoma (Stachowicz-Stencel et al. 2011). Whilst, higher TAC did not reduce signs of the metabolic syndrome in children diagnosed with either haematological malignancies or solid tumours (Krawczuk-Rybak et al. 2012). Signs of the metabolic syndrome were considered when the following parameters were abnormal: BMI, total cholesterol,

high density lipoprotein, triglycerides, fibrinogen, fasting glucose, post-prandial glucose, fasting insulin and postprandial insulin. Of note, the authors did not report the reference values for any of the parameters measured but BMI.

In conclusion, it appears from the published literature that children diagnosed and treated for cancer tend to have reduced plasma TAS, reduced endogenous and exogenous TAC and high oxidative stress. This is especially the case at diagnosis and during the first months of treatment. The reduction in TAS and TAC was mainly attributed to an increase in oxidative stress caused by the disease burden and the treatment; however, very few studies examined antioxidant intake, which might have been a major confounding factor. Additionally, the literature examining the impact of oxidative stress, TAC and TAS on clinical outcomes is heterogeneous in those measured, making it very difficult to establish whether these parameters have an impact on clinical outcome. Therefore, more research investigating these aims and taking into consideration both the weaknesses and strengths found in the published studies is warranted.

Table 1-4 Impact of antioxidants and oxidative stress on clinical outcomes

Author	Quality	Patients Dx	N, age years	Time of measurement and methods	Method			Clinical Outcomes
					Design	Variables	Outcomes	
Al-Tonbary et al. (2011) Egypt	Weak	ALL Controls (outpatient clinic of general paediatric surgery)	ALL: N=50 Age: mean±SD (range): 6.84±3.73 (1.5-12) y Controls: N=10 (matched for age and sex)	T: At diagnosis and after 5 weeksof treatment (end of induction therapy) M: Measurement of serum TAC (total antioxidant capacity): Koracevic et al 2001 Measurement of MDA (measurement of lipid peroxidation i.e. oxidative stress); Satoh K 1978 and Ohkawa et al 1979 Apoptosis DeadEnd™ Fluorometric TUNEL System (Promega Corp., Madison, WI, USA)	PCS	TAC, MDA, apoptosis	NR	Apoptosis at 5 weeks was positively correlated with MDA (r=0.858; p<0.001) and negatively correlated with TAC (r=-0.439; *p=0.041)*
Caron et al. (2009) USA	Moderate	ALL	N=88 Mean(±SD) 7.1(±3.1)y	T: from diagnosis to 3 years Diagnosis, post-induction, post-consolidation and end of therapy M: Oxidised changes in CSF PC: Miketova et al 2005	PCS	Oxidised changes in CSF	Executive functions	Scores for executive functions were higher in ALL patients in compare to normative values but did not reach significance (*p>0.05*) ↑ Oxidative stress was associated with poorer working memory, poorer organization and attention problems at the end of therapy (*p<0.05*)

				Executive function measures: The Behavior Rating Inventory of Executive Function (BRIEF): Gioia et al 2000 higher scores reflect more pathology				Oxidative stress and higher executive dysfunction at post-consolidation phase was not sig. associated
Kennedy et al. (2004)	Strong	ALL	N= 103 Age mean (±SD)y Standard-risk protocol 4.7(±2.6)y High risk protocol 10.5(±4.6)y	T: at diagnosis, 3-4 months and 6-7 months post diagnosis M: Total ascorbate analysis: HPLC analysis (Behrens et al 1987) Vitamin A and E: HPLC analysis (Bieri et al 1979) Total carotenoids Colorimetric procedure (Roels et al 1972) Dietary intake: 24h recall and FFQ Dietary analysis: Minnesota NUTRITION DATA SYSTEM (NDS) software Recommended dietary allowance (National Health and Nutrition Examination Survey 2000)	PCS	Total ascorbate Vitamins A and E Total carotenoids HDL and LDL Ch and TGA	Side effects Disease status	Association between antioxidants intake and CT toxicity: ↑vitamin intakes was associated with ↓side-effects ↑β-carotene at 3 months ↓risk of toxicity (*p=0.04;*OR: 0.87; 95% CI: 0.80-0.96) ↑Vitamin C at 3 months ↓ risk of toxicity, delays in CT treatment, days in hospital After Bonferroni's adjustment for multiple side-effect comparisons for each nutrient intake only ↑mean intakes of β-carotene ↓ risk of toxicity (*p=0.002*) Vitamin C and E intakes were associated with plasma antioxidants, but vitamin A and total carotenoid were not

				Plasma antioxidant cut-off values NR				
Kennedy et al. (2005)	Strong	ALL	N= 103 Age mean (±SD)y Standard-risk protocol 4.7(±2.6)y High risk protocol 10.5(±4.6)y	T: at diagnosis, 3-4 months and 6-7 months post diagnosis M: Total ascorbate analysis: HPLC analysis (Behrens et al 1987) Vitamin A and E: HPLC analysis (Bieri et al 1979) Total carotenoids Colorimetric procedure (Roels et al 1972) Antioxidant capacity (ORAC): Cao G and Prior R 1995 Oxidative damage (8-oxo-dG): Yarborough et al 1996	PCS	Exogenous antioxidants (vitamins A and E, carotenoids) Antioxidant capacity Oxidative stress	Antioxidant concentration and response to CT Antioxidant concentration and CT toxicity	After Bonferroni adjustment for multiple comparisons: ↑TCar/Ch was associated with ↓hospital stay at 3-4 months (*p=0.001*) ↑ Vitamin E/Ch associated with ↑need of NS (*p=0.007*) ↑plasma vitamin A was associated with both ↑QoL score (*p=0.009*)) Low plasma total carotenoid associated with ↑ toxicity
Krawczuk-Rybak et al. (2012)	Weak	Survivors: ALL, AML, NHL, HD and solid tumours Healthy controls Newly diagnosed ALL	Survivors: N=100 Age median(IQR) 12.7(8.68-16.55)y Healthy controls N=22 Age mean(±SD)	T: Survivors: mean(±SD) since cessation of treatment 5.3(±2.99)y Newly diagnosed ALL: At diagnosis During therapy M: TAS: RANDOX TAs kit (Randox, Ardmore, UK)	CC	Plasma TAS,	Signs of metabolic syndrome measured as: BMI, TCh, HDL, TG, fibrinogen, fasting glucose, post-prandial glucose,	TAS levels did not correlate with MS parameters TAS and TG were positively correlated (*p=0.02*; r=0.24)

			: 12.6(±4.79) y Newly diagnosed ALL: NR	Measurements of the Metabolic syndrome (MS): Weight, height and blood pressure: conventional techniques Abdominal obesity: Waist to hip ratio Biochemical test: automated directed methods			fasting insulin, post-prandial insulin	
Sgarbieri et al. (2006) Brazil	Weak	ALL	N= 45 Median (range) age 5(1-11) y	T: Diagnosis, induction, reinduction, and maintenance therapy M: Zn and Cu measured using atomic absorption spectrophotometry	PC	Plasma Zn and Cu concentration Dietary Zn and Cu	Prognosis of disease	In this study Zn and Cu have been investigated to find out whether these two markers can be used as prognostic factor- No correlation was found.
Stachowicz-Stencel et al. (2011) Poland	Moderate	STS NB Healthy controls	N=99 STS n= 47 median(range) 10.3(1.5-21.4) y NB n=52 median(range) 0.9 (0.2-17.8) y Healthy controls n= 30 median(ran	T: before CT and at the time of response assessment M: SOD in erythrocytes assessed according to Ransod (Randox, Crumlin, UK) procedure GPx assay in erythrocytes assessed according to Ransel (Randox) procedure Ischaemia modified albumin (IMA) assessed by	PC	Antioxidant enzymes: SOD and GPx Inflammatory marker: IMA	Response to treatment	*Authors conclude that SOD and GPx can be used as a marker to predict outcome (response).* *Issues with this:* *-Not sig diff between healthy controls and cases at diagnosis* *-Authors have not stratified the groups GR and PR at both stages diagnosis and after treatment, so how can they know that these two parameters are good markers if they only show that SOD and GSH-Px are lower after the response to treatment?*

			ge) 8.05(1.4-17.9) y	calorimetric assay using dithiothreitol (DTT). Patients stratified according to: Good response and poor response				
Stenzel et al. (2010) USA	Strong	ALL	N= 87 mean(±SD) 7.0(±3.1) y Range 3-16 y	T: at diagnosis, post-induction , post-consolidation and end of therapy M: CSF Sample phospholipids measured according to Mawatari et al. 1998 Behavioral measures: Behavior Assessment System for Children (BASC) Reynolds et al. 1992	PC	CSF phospholipids (oxidation) Sex, age at diagnosis, treatment, SES, IV-MTX dose	BASC	Sig association between IV-MTX and behavioral pathology at post-consolidation ($p<0.01$) and end of therapy Leadership skills ($p<0.05$) Younger age at diagnosis was sig associated with more aggression at the end of therapy ($p<0.01$) Higher PC oxidation fraction was sig associated with more problems in post-consolidation ($r= 0.42$; $p<0.01$)and end of therapy ($r=0.49$; $p<0.01$)

ALL: acute lymphoblastic leukaemia; ASA: Ascorbate; ASR: Ascorbyl radical; BMI: body mass index; C: cohort study; CAT: Catalase; CNS: central nervous system; CSF: cerebrospinal fluid; CS: cross-sectional study; CT: chemotherapy; DRI: dietary recommended intake; Dx: Diagnosis; EDI: estimated dietary intake; EPR: electron parametric resonance; FFQ: food frequency questionnaire; FRAP: Fluorescence recovery after bleaching; GPx: Glutathione peroxidase; HDL: high density cholesterol; ICCC: International classification of Childhood Cancer; M: method; MDA: melondialdehyde; MTX: methotrexate; NA: non-applicable; NR: not reported and/or not clear; ORAC: Oxygen radical absorbance capacity; PC: prospective cohort study; PCp: Phosphatidylcholine; RC: retrospective cohort study; ROS: reactive oxygen species; SD: standard deviation; SES: socioeconomic status; Sig: significant; SOD:Superoxide dismutase; T: time of measurement; TAC: total antioxidant capacity; TAS; total antioxidant status; TCh: total cholesterol; TEAC: Trolox equivalent antioxidant capacity; TG: triglycerides; Y: years; ↓: decrease; ↑: increase.

1.4.2 Omega-3 and omega-6 fatty acid status of paediatric cancer patients

Like antioxidants, the health benefits of omega-3 fatty acids have extensively been researched *in vitro*, in animal and in human studies, primarily in healthy subjects (van der Meij et al. 2011), but a very limited number of studies have comprehensively investigated this in children with cancer. Long chain polyunsaturated omega 3 fatty acids (LC n-3 PUFA) and omega 6 fatty acid (LC n-6 PUFA) are essential fatty acids as they are not synthesised *de novo* and they need to be supplied from the diet (Lorente-Cebrián et al. 2013). Linoleic acid, the most abundant n-6 LC-PUFAs in the western diet, is a precursor of arachidonic acid (AA). AA is a potent precursor of inflammatory markers (Lorente-Cebrián et al. 2013). Whereas, the main LC n-3 PUFAs are docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), which are found in oily fish, and α-linolenic acid, which is found in some vegetables oils, (Lorente-Cebrián et al. 2013). Both DHA and EPA are anti-inflammatory fatty acids (van der Meij et al. 2011, Lorente-Cebrián et al. 2013).

In addition, DHA and EPA have a structural role in cell membranes: DHA is the most abundant fatty acid in neural cells and they are both incorporated in phospholipids of all body cell membranes. A balance between LC n-3 PUFA and LC n-6 PUFA is necessary for homeostasis of the immune system and optimal body and brain development. This is especially the case in younger children (Gleissman et al. 2011). However, excessive LC n-6 PUFA has been associated with long term mild inflammation, which in turn may increase the risk of many chronic diseases, including CVD, cancer, rheumatoid arthritis and mental disorders (van der Meij et al. 2004; Lorente-Cebrián et al. 2013). In contrast, recent evidence show that DHA is essential for neural development (Gleissman et al. 2011) and may prevent cancer development as DHA may act as an inhibitor of malignant cell growth *in vitro* (Larsson et al. 2004, Greene et al. 2011). Additionally, due to their anti-inflammatory properties, EPA and DHA are thought to protect against CVD, cancer and some autoimmune conditions, including rheumatoid arthritis (Lorente-Cebrián et al. 2013).

Although, LC n-3 PUFAs have anti-inflammatory properties and thus they are beneficial for healthy individuals in the prevention of cancer (Greene et al. 2011, Lorente-Cebrián et al. 2013), there are many factors at interplay in children treated for cancer that affects the immune system. Also, the degree to which DHA and EPA suppress inflammation in this population and whether this is beneficial is yet to be established. As discussed in section 1.3 the immune system is affected by the disease and the use of glucocorticoids, chemotherapy and radiotherapy as well as the use of non-steroidal anti-inflammatory drugs (NSAID). All these factors have dichotomous effects in the immune system; anti-inflammatory and pro-inflammatory.

The use of immunosuppressants drugs, primarily glucocorticoids, on the one hand inhibits the immune system by inhibiting the transcription of genes that code for the up-regulation of inflammation (AP-1 and NF-κB). On the other hand, dexamethasone might interfere with the metabolism of PUFA by inhibiting the endogenous synthesis of LC n-3 PUFA to EPA and DHA (Marra et al. 1986). The ratio of AA/DHA (and AA/EPA) and the subsequent inflammatory markers would increase. Methotrexate, a folate antagonist drug, causes bone marrow toxicity, which results in a reduction in leukocyte synthesis and an increased susceptibility to infection. In contrast, its toxic effects on healthy cells induce an inflammatory response (Rang, et al. 2003). EPA and DHA have also been shown to inhibit NF-κB, which might further inhibit inflammation (Calder 2010) (see figure 1.7 for detail). In contrast, the disease itself and the toxic effects of the chemotherapy and radiotherapy stimulate an anti-inflammatory response due to the destruction of normal body cells (Lorente-Cebrián et al. 2013, Larsson et al. 2004).

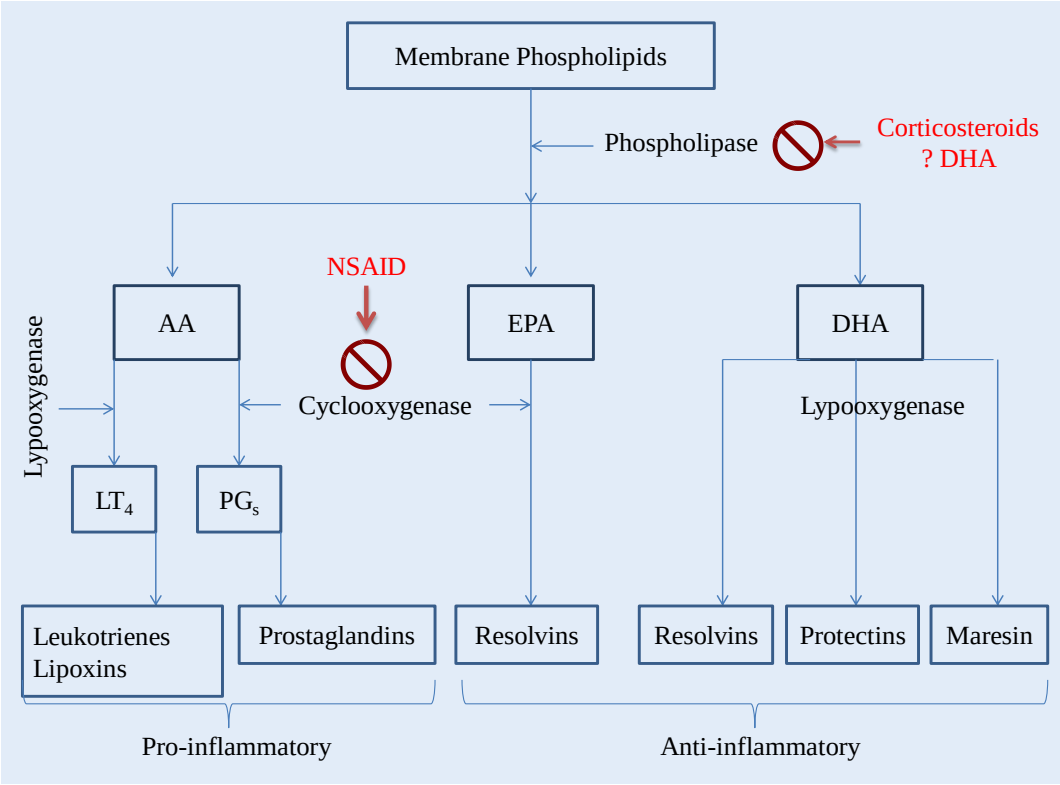

Figure 1.7 Effects of Corticosteroids, NSAID and DHA on inflammation

Evidence regarding the protective role of LC n-3 PUFA in cancer prevention and treatment is emerging (Greene et al. 201, van der Meij et al. 2011). However, there is limited epidemiological data reporting plasma PUFA status in children with cancer and no studies have been published investigating the efficacy of PUFAs supplementation in this population. Of note, animal and human studies have demonstrated that PUFAs, especially DHA, can be used as vectors for anti-cancer agents, and consequently being more specific and target cancerous cells (Wang et al. 2013). This has been attributed to their lipophilic nature and their readiness to be incorporated into cell membranes, especially from tumour cells (Gleissman et al. 2011). Furthermore, a recent systematic review reported that there is a large body of evidence published investigating the efficacy of LC n-3 PUFA, DHA or EPA supplementation in adults diagnosed and treated for cancer (van der Meij et al. 2011). Evidence reporting plasma PUFA in cancer patients is contradictory. Only one epidemiological study found in the literature investigated the profile of PUFA in 12

children diagnosed with CNS, bone and abdominal tumours and having received at least one month of chemotherapy. Plasma PUFA was compared with a healthy matched control. Plasma EPA and α-linoleic acid was lowered than in the control group; however, plasma DHA was not significantly reduced (de la Torre Aguilar et al. 2012). In contrast, the plasma DHA and EPA of 47 adults diagnosed with NHL were found to be lowered than the healthy controls (Cvetkovic et al. 2010). Notably, none of the two studies reported significant differences in lipid intake (Cvetkovic et al. 2010; de la Torre Aguilar et al. 2012). With regards to the efficacy of LC n-3 PUFA supplementation in adults with cancer, the systematic review concluded that supplementation, given as ONS, capsules or NG-feeding, increased plasma DHA and EPA in most studies. It also appeared to have beneficial effects on the clinical outcomes body weight and quality of life. All the other clinical outcomes measured; morbidity, mortality and Karnofsky Performance Status, were inconsistent (van der Meij et al. 2011).

In summary, it appears that supplementation of LC n-3 PUFA, especially DHA and EPA, might be beneficial in adults with cancer; however, there is very limited published evidence in children. Before supplementation is implemented in children with cancer, more research looking at the following is urgently warranted: epidemiological studies assessing plasma PUFAs status and the impact of PUFAs on clinical outcomes, including: morbidity, mortality, side-effects, infections and survival. Also, measurements of growth and development should be monitored. Finally, well designed RCT investigating the effectiveness of different forms of supplementation, in which doses of PUFAs are taken into consideration, are justified.

1.5 NON-ANTIOXIDANT MICRONUTRIENT STATUS OF PAEDIATRIC CANCER PATIENTS

In addition to plasma vitamins and minerals with antioxidant function, there are other micronutrients, which reflect nutritional status and are essential to maintain healthy biological functions. These are also likely to be affected by the cancer and its associated treatment. Thus this section will review published studies of paediatric cancer patients reporting micronutrient status.

1.5.1 Vitamin D

Results from phase I of this project showed that a large percentage of children and adolescents diagnosed and treated for cancer in South East Scotland were classified as either vitamin D (25(OH)D) deficient (25(OH)D <25nmol/L) or insufficient (<50nmol/L). At this stage of the study, there were 16 patients with available vitamin D results. Of these, 5 (31%) were deficient and 7 (44%) insufficient at the time of diagnosis, thus a total of 75% (12/16) had 25(OH)D inadequacy (levels below insufficiency) at diagnosis (Paciarotti I 2013). Although these were preliminary results, it showed that vitamin D inadequacy in this population was much higher than that reported in healthy children from the UK (19%) (Department of Health 2012). Additionally, a pattern appeared to emerge whereby solid tumours showed higher levels of 25(OH)D inadequacy (44%) than children diagnosed with haematological malignancies (31%). However, 25(OH)D levels decreased in the solid tumours and increased in the haematological group during the course of treatment. This was attributed to the use of steroids and to the large percentage of obesity seen in children diagnosed with haematological malignancies, mainly ALL (Paciarotti I 2013).

As a consequence of these findings, a systematic review was planned at the start of phase II. The main aims of this were to establish worldwide vitamin D levels in paediatric oncology patients at different stages of the disease and treatment, to investigate the possible causes of vitamin D inadequacy in this population and to finalise by comparing the findings from the systematic review and those obtained from paediatric oncology patients from SE Scotland. This will be fully described in chapter III, section 2.2.

1.5.2 Other micronutrients

To date, there is not a single published study investigating the plasma concentration of B vitamins, vitamin K and folic acid in paediatric cancer patients. However, there is a paucity of evidence investigating vitamin B12 (Carmel and Eisenberg 1977, Carmel 1975, Coltman et al. 1975, Vlasveld LT. 2003) and folic acid in adults diagnosed and treated for cancer (Carmel and Eisenberg 1977, Vlasveld LT. 2003). In addition, no study has investigated iron status either by measuring total iron binding capacity in acute patients, ferritin after the completion of therapy or ferritin in acute patients to detect iron overload, which threshold has been set at >1000 μg/L (Henter et al. 2007, Schrappe & Pieters 2004).

The few studies reporting plasma vitamin B12 (cobalamin) in adults diagnosed and treated for cancer have shown that although most patients have levels within the normal range, a significant percentage has both low and high levels. For instance, Carmel and Eisenberg (1977) investigated plasma vitamin B12 and transcobalamin, which is a vitamin B12 binding protein, in 139 patients diagnosed with solid tumours. Of these, 69% did not present with any vitamin B12 anomalies; however 11% had elevated levels and 10% had low levels. Similarly, Vlasveld (2003) found that of the 32 patients diagnosed with multiple myeloma who had also undergone treatment, 66% had normal plasma vitamin B12 levels, whilst 28% and 6% had low and elevated levels respectively. Both studies (Carmel & Eisenberg 1977, Vlasveld 2003) also investigated plasma folic acid, which was found to be within the normal range.

In health, serum ferritin is a useful marker for the assessment of iron stores. In paediatric cancer patients, particularly in ALL, the interpretation of ferritin as an iron marker is complicated by elevation due to leukaemic synthesis of ferritin by blasts, acute phase response, or rare macrophage activation syndromes such as haemophagocytic lymphohistiocytosis (HLH), which is defined by fulfilment of five out of eight criteria: ferritin>500μg/L, fever, splenomegaly, cytopenias, hypertriglygeridemia or hypofibrinogenemia, haemophagocytosis, low NK cell activity and/or raised soluble CD25. Identifying iron overload accurately, especially in the context of ALL has clinical importance. Ferritin levels >1,000 μg/L are

considered indicative of iron overload, corresponding to a level at which chelation therapy is advised like in chronic haemolytic anaemia (Henter et al. 2007, Schrappe & Pieters 2004). However, no studies have assessed this in the context of nutrition.

From this limited body of evidence two conclusions can be made. Firstly, it appears that plasma vitamin B12 might be affected in cancer patients. Secondly, it is clear that more research is urgently warranted in order to elucidate whether cancer and its associated treatment affects the micronutrient status of this population.

1.6 NUTRITIONAL MANAGEMENT IN CHILDREN AND YOUNG PEOPLE WITH CANCER: CURRENT PRACTICE

The nutritional management of hospitalised children and adolescents, including those diagnosed with and treated for cancer, is vital to reduce the risk of malnutrition and allow optimal growth and development. This in turn is thought to reduce hospital stay and the risk of morbidity and mortality (Donaldson et al. 1981, Mejía-Arangure et al. 1999, Sala et al. 2004, Agostoni et al. 2005). Despite this the ESPGHAN committee, based on a report from the council of Europe published in 2002 (Beck et al. 2002), concluded that there were major issues across Europe related to the nutritional management of paediatric patients, including cancer patients (Agostoni et al. 2005b). For instance, routine nutritional risk screenings and assessments were often not implemented, the responsibility for food services and nutritional care was not clearly assigned and members of staff were not appropriately trained regarding clinical nutrition. Additionally, food intake was not appropriately recorded and allocated meal times were inflexible (Beck et al. 2002, Agostoni et al. 2005).

Prior to the ESPGHAN report, an audit exploring the nutritional management of children and adolescents admitted and treated in the Haematology/Oncology ward from the RHSC Edinburgh, was performed. From this audit, the following issues were raised: (i) there was not a systematic approach to screening for malnutrition and the referrals were not designated to one specific team, thus this was done on an ad hoc basis creating confusion between members of staff; (ii) 70% of growth charts were incomplete or were missing and most children did not have weights and heights taken; (iii) the mean length of time between diagnosis and referral was 2.7 weeks and by that time the patients had already lost 7.6% of total body weight. Furthermore, by the time the patients were seen by a dietitian a further 5.9% weight loss had occurred (total weight loss 13.5%) (Holt 2003). These reports highlighted the need for introducing a systematic approach to patients screening and the need for multidisciplinary nutrition support teams. Thus, at present a screening tool specifically designed for the general paediatric population is in used in the RHSC Edinburgh; the Paediatric Yorkhill Malnutrition Score (PYMS). Additionally, the Subjective Global Assessment (SGA), originally designed by Detsky and co-authors

(1987), is also currently being used for the assessment of nutritional status and for the implementation of nutritional support; despite the publication of a recent updated model (see figure 1.8) advising on the nutritional management of children and adults (BDA 2012), which proceeds the general nutritional management pathway (Elia 2005). Although the above changes have gradually been implemented, nutritional screening and management remains difficult in this population and the reasons for this will be discussed in this section.

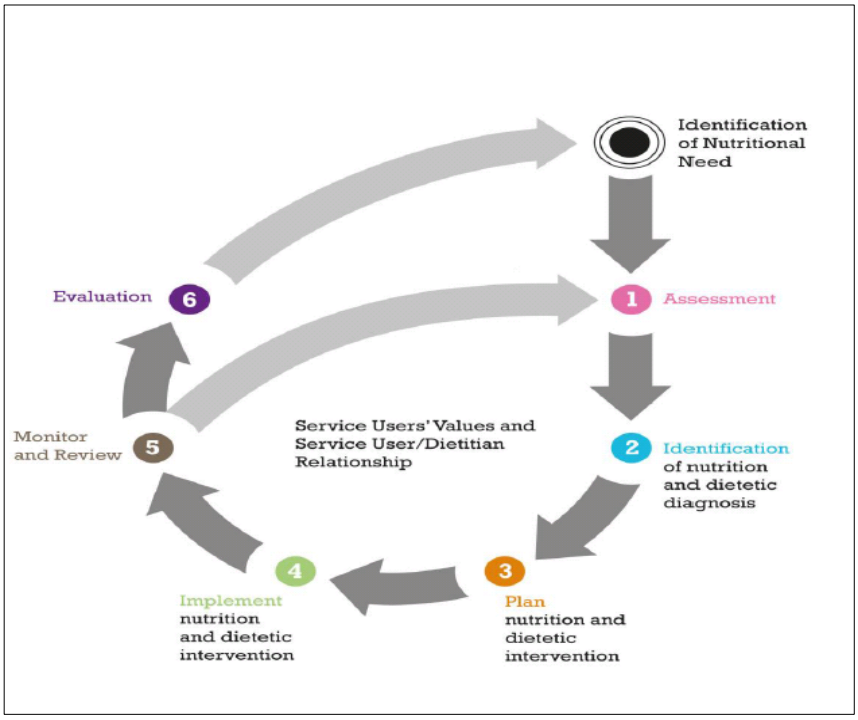

Figure 1.8 Model and process for Nutrition and Dietetic Practice (obtained from BDA 2012).

1.6.1 Where are we with the nutritional screening tools?

Nutritional screening is fundamental to identify patients who are malnourished or at risk of developing malnutrition to subsequently be referred to a nutrition specialist team if necessary (BDA 2012). Unlike adults, at present there is no universally accepted screening tool to identify the risk of malnutrition specifically designed for the unique paediatric oncology population (Attard-Montalto et al. 1998, Sermet-Gaudelus et al. 2000). However, four non-disease specific nutritional screening tools have been developed to identify both children at risk of undernutrition and

undernourished and all are widely used in hospitals: the Screening tool for Assessment of Malnutrition in paediatrics (STAMP) developed and used in England (McCarthy et al. 2012); the Paediatric Yorkhill Malnutrition Score (PYMS) developed and used in Scottish hospitals (Gerasimidis et al. 2010); the Screening tool for Risk on Nutritional status and Growth (STRONG kids) developed and used in the Netherlands (Hulst et al. 2010). Finally, the Simple Paediatric Nutrition Risk (PNRS), which was developed in France and designed to be used in hospitalised children (Sermet-Gaudelus et al. 2000). These tools are simple and easy to use tools and are based on severity of disease, percentage weight loss, BMI and dietary intake with some variations between each screening tool. Nevertheless, a number of issues can be raised when these are applied to paediatric cancer patients: (i) all tools either included a very small sample of children with cancer (PYMS: 2.4% (38/1571); STRONG kids: 3% (12/296); PNRS: 4% (12/296)), or the diagnosis was not mentioned (STAMP: n=360 medical and surgical) making them unlikely to be representative and applicable to this unique population; (ii) Every child diagnosed with cancer and receiving any of the cancer therapies would be considered at high risk of undernutrition, which could increase the number of false referrals. (iii) Finally, all tools screen for undernutrition, whilst overnutrition is overlooked.

Attempts have been made to either develop disease specific nutritional tools or to validate current ones. For instance, the Subjective Global Nutritional Assessment (SGNA), which is an adaptation of the SGA originally designed by Detsky and co-authors (1987), was validated in children who were having major abdominal and thoracic surgery (Secker and Jeejeebhoy 2007). This tool was later proved to be too complicated and time consuming to use in clinical practice (Joosten & Hulst 2011). Additionally, the STAMP has been validated in children with a spinal cord injury (n=62) (Wong et al. 2012) and the STAMP, PYMS, STRONG kids and PNRS in children diagnosed with inflammatory bowel disease (n=46) (Wiskin et al. 2012). Therefore it can be seen that none of them have been validated in the paediatric oncology population. Of note, a few have been performed in adults with cancer undergoing specific treatments (Ferguson et al. 1999, Bozzetti 2009).

1.6.2 Nutritional assessment of paediatric cancer patients

In section 1.2.1 both the methods of nutritional assessment and published UK standards to assess the nutritional status of healthy children and young people were discussed in depth. Thus, this section will focus on the practical aspects and current practice when assessing the nutritional status of paediatric cancer patients following the model proposed by the BDA (2012).

1.6.2.1 Assessment of growth and body composition

At present, a standard measure that best determines growth in children with cancer is lacking; however, most physicians worldwide and in the Edinburgh RHSC rely on BMI, weight and linear growth patterns (Sala et al. 2004). Additionally, although reliable methods exist to assess the body composition in both healthy children and children with cancer, these are not routinely performed in paediatric cancer patients. Also, they all have its strengths and limitations.

BMI is the assessment of growth most widely used in clinical practice. BMI is easy to calculate from weight and height, does not require high level of skills, it is inexpensive and internationally recognised (Cole et al. 1995; Sala et al. 2004; Cole et al. 2007; Brinksma et al. 2012); however, it can be misleading in many children with cancer (Sala et al. 2004). As it has previously been discussed the weight of a solid tumour in infants and younger children can account for up to 10% of total body weight, the effects of many chemotherapeutic agents can cause oedema and glucocorticoids stimulate both proteolysis and lipolysis (BNF 2009). This causes a reduction in FFM and an increase in abdominal FM, which can lead to protein energy malnutrition (Brennan 1998, Sala et al 2004, Sala, Pencharz & Barr 2004, Collins et al 2010). As BMI measures body surface only, none of these factors are taken into consideration and many malnourished paediatric cancer patients can either be undiagnosed or misdiagnosed (Sala et al. 2004).

MUAC and TSF are not routinely performed in clinical practice in the RHSC, Edinburgh. These two measurements are inexpensive and non-invasive (Norton & Olds, 1996); however a high level of skill, which is achieved by training, is needed to reduce intra- and inter-error of measurement by the anthropometrists in order to

obtain accurate measurements. Despite this, these two measurements have the following advantages over BMI in this population; it allows for the calculation of FFM and FM, providing more information regarding body composition changes and, it is generally less affected by oedema and tumour mass (White et al. 2011, Garófolo et al. 2005, Oguz et al. 1999)

Like MUAC and TSF, BIA is not used routinely to estimate the body composition of paediatric cancer patients from the RHSC, Edinburgh. Although it is non-invasive, inexpensive and painless and thus a potential practical tool in clinical practice, its use has been argued due to a lack of a standard TBW equation from which FFM% and FM% can be calculated. At present, there is only one TBW equation developed from a paediatric cancer cohort (Brennan et al. 1997). The authors attempted to validate a TBW equation by comparing measurements obtained from BIA, TSF and deuterium water dilution from a cohort of 40 children diagnosed with any malignancy. BIA was found to have a similar precision to the deuterium water dilution method; however it had wide limits of agreement (-2.46 to 4.06), which was attributed to the small sample size (Brennan et al. 1997) and perhaps due to the diversity in cancer diagnosis included. Dramatic changes in weight and shifts in fluid distribution also occurred as a result of the metabolic response to cancer and its treatment, which altogether may lead to inaccurate readings (Kyle et al. 2004a, Kyle et al. 2004c). Finally, reference values of FFM% and FM% of healthy children from which to compare the data are limited (Fomon et al. 1982, Laurson et al. 2011, Wells et al. 2012) and not without limitations. The main issues identified are the following: age range is not always available from birth to adulthood (19-20 years) (Fomon et al. 1982; Laurson et al.2011; Wells et al. 2012); the reference value was developed from only one subject (Fomon et al. 1982); FFM% and FM% were not always obtained from gold standard methods and some provided reference values only for FM%, but not for FFM% (Laurson et al. 2011). Despite these limitations, the use of BIA might be of value in prospective cohort studies where the aim is to investigate changes in body composition as a result of cancer and cancer therapy.

Presently, none of the discussed methods taken in isolation appear to provide with an accurate picture of growth and body composition in paediatric cancer patients during

the course of treatment. However, if taken in conjunction at diagnosis, over the course of treatment and post-treatment, they perhaps would help to elucidate the nutritional status changes that occur in this population. Thus, allowing for a more comprehensive nutritional assessment and consequently more tailored nutritional support.

1.6.2.2 Assessment of dietary intake

The dietary intake assessment of paediatric oncology patients in clinical practice and in research is performed by using several methods and these vary depending on the objective and feasibility of the assessment. The methods most commonly used are, 24-hour dietary recall (Delbecque-Boussard et al. 1997, Delbecque-Boussard et al. 1997, Kennedy et al. 2004a), diet histories (Bond et al. 1992, Carter et al. 1983, Todorovic.& Micklewright 2007) and three to seven days diet diaries (unweight or weighted food records) (Brinksma et al. 2014, Burrows et al. 2010, Skolin et al. 1997). All of these methods carry strengths and limitations with them, but the most common ones in children are misreporting and under-reporting (Burrows et al. 2010) and micronutrients inaccuracies (Kennedy et al. 2005). A 24-hour dietary recall involves the recording of everything a subject has eaten and drunk the day before in as much detailed as possible. It is considered quick, simple and tends to cause minimal burden to patients or carers. Also, in healthy children aged between 4-11 years it has proved to be the most accurate when this is compared to the gold standard (doubly labeled water) (Burrows et al. 2010). However, its use for pre-school children (3-4) has been questioned as it is been shown to overestimate energy intake (Reilly et al. 2001). Unlike healthy children, children with cancer experience changes on dietary habits as a result of the disease and the treatment induced side-effects; thus, this method might not show these changes (Selwood et al. 2010). Nevertheless, its use becomes more reliable when it is used on alternative occasions to assess long term dietary patterns. Consequently, it is the preferred tool for population based prospective studies (Thomas & Bishop. 2009, Burrows et al. 2010, Jain et al. 1980). A diet history is generally the method of choice by dietitians to assess the dietary intake of an individual in clinical practice (Bingham 1987). The interviewer not only asks about the dietary intake of the previous 24 hours, but also

asks about usual eating patterns (Thomas & Bishop 2009, Burrows et al. 2010, Jain et al. 1980). This method requires high level of expertise, is time consuming and difficult to standardise (Bingham 1987). For that reason it is often not used for research purposes, yet it has been proved to be the most accurate for the assessment of teenagers' energy intake (Burrows et al. 2010). Finally, unweight and weighted diet diaries require the child or carer to record and weight respectively everything that is eaten and drunk over a period of time (Bingham 1987). This method is popular in clinical trials involving healthy subjects and in children under the age of 4 (Burrows et al. 2010); but, in prospective cohort studies involving children diagnosed and treated for cancer, this method is too troublesome and diet diaries are generally not returned (Paciarotti 2013).

1.6.3 Nutritional Support: current practice

Nutrition support aims to prevent, maintain or restore body stores to allow for optimal growth and development (Selwood et al. 2010, Bauer et al. 2011), yet in cases of severe disease and intensive treatment, the aim might be to minimise wasting (Bauer et al. 2011). Appropriate nutritional support also aims to improve tolerance of therapy by reducing treatment induced side-effects and, reduce the risk of infections, to ultimately improve clinical outcomes and quality of life (Bauer et al. 2011, Selwood et al. 2010). However, the nutritional support of paediatric cancer patients presents many challenges due to the large spectrum of cancer diagnoses, the intensive treatments and the lack of specific nutritional guidelines specific to this population. In addition to these, the estimation of many nutrients had to be extrapolated from the dietary reference values from the adult population as these were not available for children and young people (Department of Health 1991, SACN 2011). Specific nutrient requirements for the paediatric oncology population is not available either (Bauer et al. 2011), making it difficult to estimate accurate energy and micronutrient requirements to provide appropriate nutritional support (Todorovic & Micklewright 2007, Department of Health 1991). Recently, new dietary reference values have been published and this includes reference data for healthy infants, children and young people, which were unavailable before (SACN

2011). These are not currently used as a reference for the calculation of nutrient requirements in the oncology population of SE Scotland yet.

Nutritional support is defined as "the administration of nutrients in place of or in addition to that provided by normal eating" (Jones et al. 2010). The route of feeding is decided by either a dietitian only or the multidisciplinary team following a thorough clinical and nutritional assessment. There are two routes: the enteral route and the parenteral route, but sometimes a combination of the two might be needed (advanced nutritional support). The enteral route involves the alimentary tract and the parenteral route is the direct administration of nutrients to the blood via intravenous feeding, thus it bypasses the alimentary tract. Enteral nutrition includes oral nutrition support (ONS), but most often indicates the use of enteral tube feeding (ETF) like a nasogastric (NG), nasojejunal (NJ), gastrostomy (PEG) or jejunostomy (PEG-J) tube feeding (NICE 2006).

A paucity of evidence has examined the use of nutritional support in paediatric oncology patients (Brinksma et al. 2014, Paciarotti I 2013), even though malnutrition, especially undernutrition, has been reported to be an issue for a long time (Sala et al. 2004). Also, most studies have focused on the effectiveness of specific nutritional treatments on the nutritional status of this population or compared the effectiveness of EN and PN; as described by a recent systematic review (Jones et al. 2010). For the purpose of this review only studies examining the use of nutritional support in cohort studies will be discussed. Only two were found. A retrospective cohort study performed in SE Scotland, in which data from 168 paediatric cancer patients was available, showed that this population had high needs for nutritional support. 44% (74/168) required any form of nutritional support. Of these 57/74 (77%) required ETF, 40/74 (54%) ONS and 32/74 (43%) PN. Additionally, the diagnoses that most required nutritional support were AML and neuroblastoma (75%) followed by malignant bone tumours (67%) and CNS malignancies (57%). No significant difference in weight SD was obtained before and after nutritional support, indicating that nutritional support was effective; however, the author highlighted that these results could have been due to the large number of missing data. Similarly, Brinksma et al. (2014) in a prospective cohort study (1 year follow up) performed in

the Netherlands, in which 133 paediatric cancer patients were included, reported that 45% of these patients required NG feeding at some point during the first year of treatment. Interestingly, only one child required PEG feeding and none of the 133 children required PN (Brinksma et al. 2014). The study reported an increase in weight and FM% with a tendency to obesity, which was attributed to low physical activity rather than energy intake. Of note, neither the effectiveness of nutritional support nor the types of cancers requiring more nutritional support was reported; yet these were not part of the study's objectives.

The most important conclusion in regards to the use of nutritional support in paediatric cancer patients is the need for more UK and worldwide epidemiological studies that not only assess the prevalence of patients needing nutritional support, but also investigate its efficacy by assessing measurements of body composition, tolerance of nutritional therapy, quality of life and clinical outcomes (EFS, deaths, etc.).

1.7 OVERALL STUDY AIMS

- To systematically review the prevalence of malnutrition in paediatric cancer and to establish whether there is an association between nutritional status and clinical outcome.
- To systematically review the prevalence and possible causes of vitamin D inadequacy of paediatric cancer patients.
- To identify patterns of change in the nutritional status of paediatric cancer patients throughout the course of the disease and treatment;
- To investigate clinical and nutritional parameters that may contribute to the development of malnutrition.
- To investigate the antioxidant status, oxidative stress and polyunsaturated fatty acid status of paediatric cancer patients.
- The long term goal of this project is to provide the basis to perform a quality improvement project of the nutritional care of paediatric cancer patients in SE Scotland.

CHAPTER II

2. SYTEMATIC REVIEWS ON NUTRITIONAL ISSUES IN CHILDHOOD CANCER

2.1 SYSTEMATIC REVIEW OF THE PREVALENCE OF MALNUTRITION IN PAEDIATRIC CANCER: EFFECTS OF CANCER AND ITS TREATMENT ON NUTRITIONAL STATUS

2.1.1 Introduction

Cancer is the most common disease-related cause of childhood death in the Western World (Cancer Research UK 2012b). Nonetheless, five-year survival rates have improved considerably in the last 40 years (Cancer Research UK 2012b) after the implementation of more intensive treatments, particularly in developed countries (Herbst et al. 2006). Consequently, attention is shifting to the reduction of treatment-related sequelae during and after therapy. However, most children with cancer live in low- and middle-income countries accounting for 94% of all deaths in people aged 0-14 years (Pritchard-Jones et al. 2013). New cancer therapies are not easily accessible in these countries, whereas attention could focus on nutritional management to raise standard of care in a feasible manner (Pritchard-Jones et al. 2013).

Malnutrition, defined as undernutrition, overnutrition or poor growth (Brennan 1998, BAPEN 2010, Anon.2006b). is a major concern in paediatric cancer. It is thought to increase the risk of morbidity (van Eys et al. 1980), mortality (Sala et al. 2004, Lange et al. 2005, Reilly et al. 1994, Butturini et al. 2007), early relapse (Viana et al. 1994) and the number of complications during treatment (Anon.1998, van Eys 1979a). Additionally, a recent SIGN guideline highlighted that long-term childhood cancer survivors might be at higher risk of developing the metabolic syndrome, cardiac complications and may have a reduced peak bone mass due to related treatment side-effects (Wallace et al. 2013). This is exacerbated by malnutrition, as obesity is an independent risk factor for cardiovascular disease (Hubert et al. 1983, Bauer et al. 2011).

Malnutrition in paediatric cancer has long been recognised (van Eys 1979a, Brinksma et al. 2012, Sala et al. 2012, Jaime-Pérez et al. 2008, Odame et al. 1994, Bauer et al. 2011, Reilly 2009a, Antillon et al. 2008), yet, its management remains variable (Sala et al. 2004, Brinksma et al. 2012, Reilly et al. 1999, Brouwer et al.

2007), with many malnourished children unrecognised and consequently not treated (Agostoni et al. 2005a, Anon.2010). To date, several narrative reviews (Sala et al. 2004, Bauer et al. 2011, Reilly 2009a, Brouwer et al. 2007) and a systematic review (Brinksma et al. 2012) have investigated undernutrition in paediatric cancer. The narrative reviews provide a summary of the prevalence of undernutrition and evidence for correlations with clinical outcomes in paediatric cancer. The systematic review reports the prevalence of undernutrition, but not overnutrition, and explores the aetiology of undernutrition; the literature search finished in September 2010. The prevalence of undernutrition varies considerably between different studies; ranging from 5-10% in children with acute lymphoblastic leukaemia (Sala et al. 2004, Collins et al. 2010, Brinksma et al. 2012, Barr et al. 2011) to 50% in those diagnosed with neuroblastoma (Green et al. 2008). The associations between undernutrition and clinical outcomes also remain unclear; with some arguing that undernutrition is associated with worse outcomes (Sala et al. 2012, Donaldson et al. 1981, Lobato-Mendizábal et al. 1989, Mejía-Aranguré et al. 1999) and others that such associations do not exist (Weir et al. 1998, Pedrosa et al. 2000). Finally, obesity is likely to occur in survivors of acute lymphoblastic leukaemia, who have been treated with corticosteroids (Robison and Bhatia 2003) and in children diagnosed with craniopharyngioma; (Iughetti and Bruzzi 2011); however, at present no systematic review has been published to support these findings.

To reduce nutritional related side-effects, it is first essential to recognise the epidemiological picture of malnutrition in paediatric cancer. Thus, the study aims were: (i) to report the prevalence of malnutrition in paediatric cancer, (ii) to identify those at risk of developing both undernutrition and overnutrition during and after the completion of treatment, (iii) and to establish whether there are associations between malnutrition and clinical outcomes.

2.1.2 Methods

A protocol was designed a priori. The process and reporting of this systematic review was performed according to the Cochrane Collaboration and following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al. 2009).

2.1.2.1 Outcome data

The primary outcome was:

i. The prevalence of malnutrition (undernutrition and overnutrition) in children diagnosed and treated for cancer; expressed as body mass index (BMI).

The secondary outcomes were:

i. Studies reporting malnutrition identified with the following anthropometrical measurements; weight, height, mid-arm circumference (MUAC) and triceps skinfold (TSF).

ii. Studies reporting prevalence of malnutrition at different stages of the disease; at diagnosis, during therapy and at the end of treatment.

iii. Studies investigating the relationship between nutritional status at diagnosis and clinical outcome.

2.1.2.2 Eligibility criteria and search strategy

The eligibility criteria included:

i. Studies investigating children and young people, aged <18 years and diagnosed with a first time cancer according to the International Classification of Childhood Cancer, third edition (ICCC-3) (Steliarova-Foucher et al. 2005).

ii. To minimise the risk of bias there was no restriction on follow up period, however we excluded studies, which only investigated the nutritional status of childhood cancer survivors. Additionally, studies including patients with second cancers or treated palliatively from diagnosis were excluded.

Electronic searches of the English language were performed (January 1990-February 2013) using the Cochrane Library, MEDLINE (via EBSCOhost), CINAHL (via EBSCOhost) and PUBMED to identify systematic reviews, Randomised Controlled Trials (RCT), observational studies, case control studies and letters to the editor. The reference list list of all relevant articles, narrative reviews and private collections was also examined. The initial search strategy identified the following keywords and subject heading searches (MeSH); "paediatrics", "cancer", "cancer treatment", "malnutrition" and "nutritional assessment" and adaptations for British and American English were made for all searches.

2.1.2.3 Study selection, quality assessment and data extraction

Titles and abstracts from the combined searches were reviewed by two researchers independently (RRI, JMcK). In cases of disagreement, an independent advisor (DW) made the final decision. Evidence was critically appraised independently by two researchers (RRI, IP) employing a standard methodological tool; the Critical Appraisal Skills Programme (CASP). The CASP tool has been recommended for epidemiological studies, public health and evidence based practice (Sanderson et al. 2007). The CASP comprises an assessment of: (i) Contextual information including the study objectives, study design and the patient's characteristics; (ii) Potential selection bias including inclusion and exclusion criteria, clear patient selection and an assessment of validity, reliability and accuracy of techniques used; (iii) Outcome measures including reference values; (iv) statistical analysis employed and (v) a reporting of results and control for confounding factors. Each section was classified as "yes" if the authors covered the questions, "can't tell" if it was not reported or "no" if it was not covered or considered at all. A quality rating of "strong", "moderate" or "weak" was applied to each study; where "strong" was given when the study met all areas of the CASP criteria, "moderate" when there was one weak area and "weak" when ≥ two weak areas were identified. A third independent reviewer (DW) made the final assessment and in case of disagreement a final decision was made by consensus (RRI, IP, DW). A meta-analysis of selected studies investigating the association between malnutrition and outcome was planned to be performed a

priori. Finally, the median and the range percentage of the prevalence of malnutrition were calculated for comprehensibility of the results.

2.1.3 Results

2.1.3.1 Study Selection and characteristics

46 studies met our eligibility criteria (figure 2.1). Of these, 26 were prospective cohort studies, 19 retrospective cohort studies and one cross-sectional study. Twenty-nine studies investigated malnutrition in children diagnosed with haematological malignancies and acute lymphoblastic leukaemia was considered in all studies. Thirteen considered solid tumours, two brain tumours and eight studies considered children diagnosed with cancers included in the ICCC-3. Six studies investigated more than one cancer type. For differences between developed and developing countries, see figure 2.2.

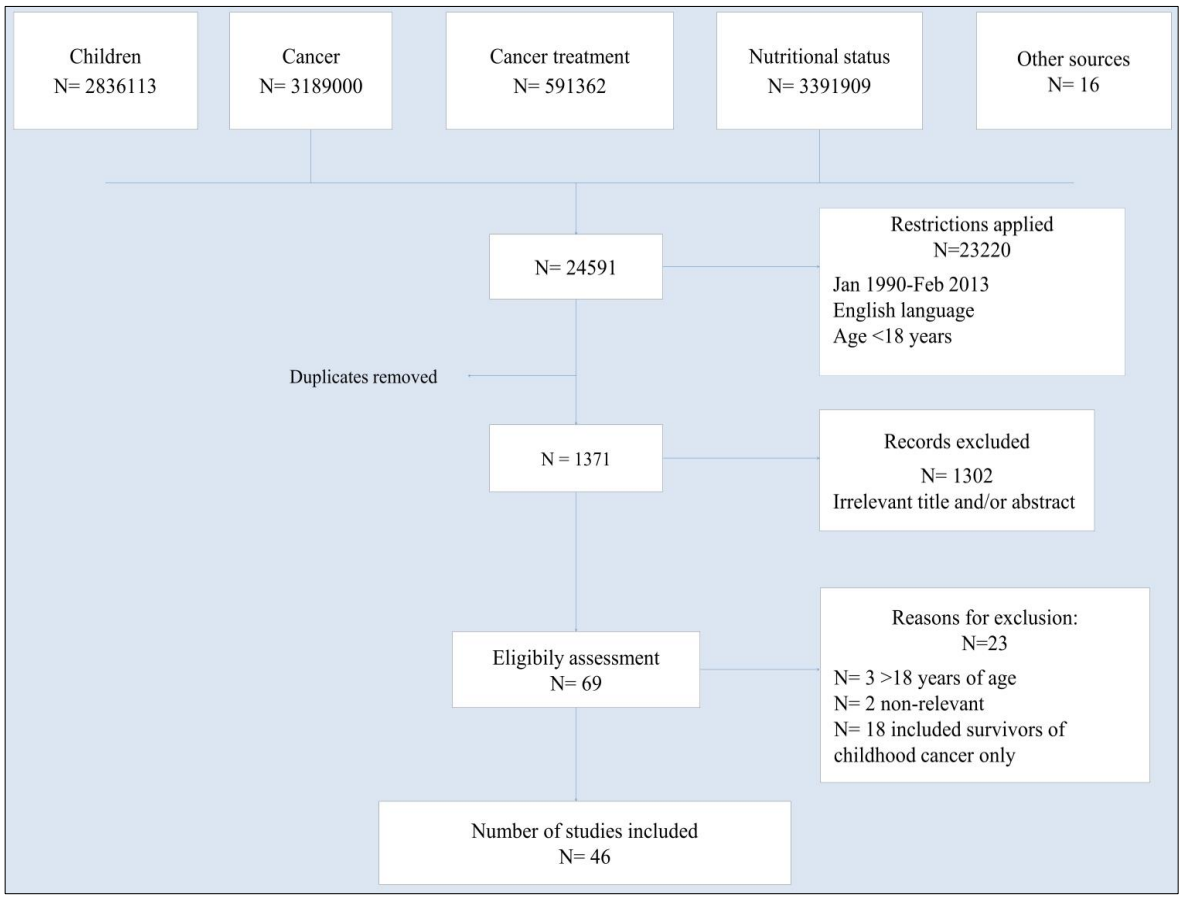

Figure 2.1 Flow diagram of studies identified, screened and selected

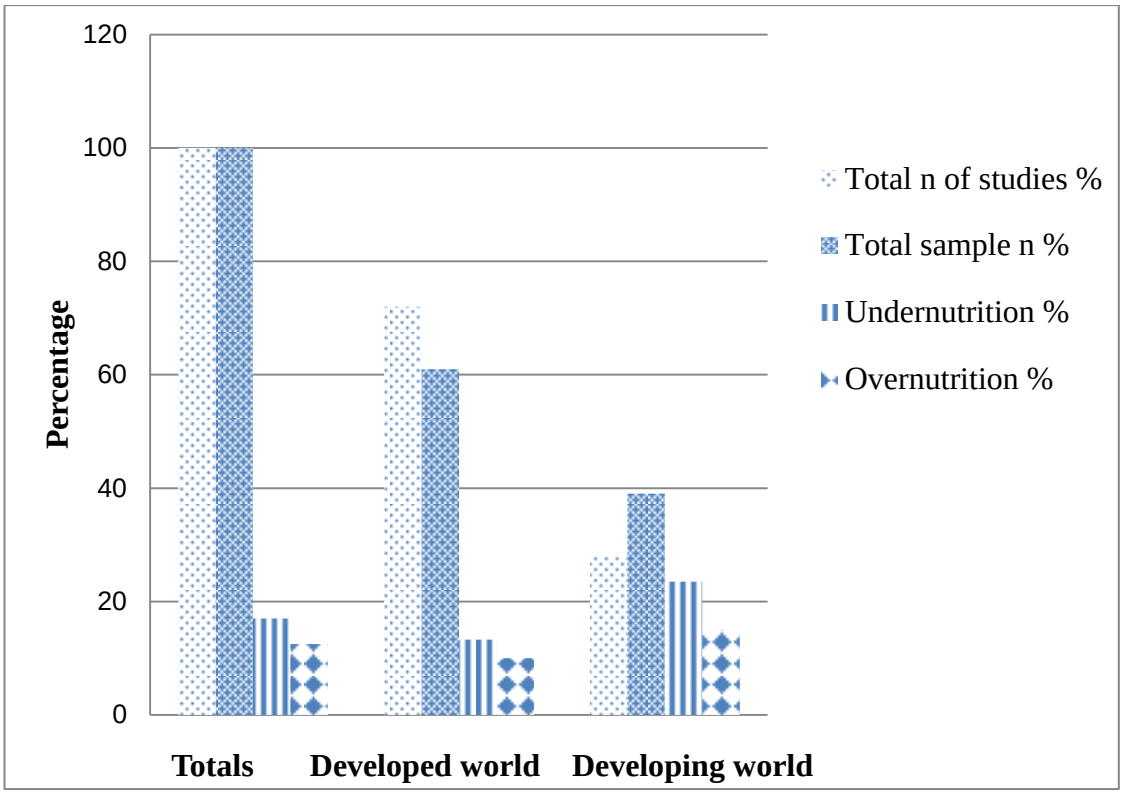

Figure 2.2 Comparison of the total number of studies published (%), total sample size (%), and the prevalence of undernutrition and overnutrition.

Total number of studies (46); number of studies published in the developing world (13); total number of studies published in the developed world (33); total number of subjects included in all studies (8734); sample size from the developing world (3411) and sample size from the developed world (5323)

2.1.3.2 Methods used to identify malnutrition.

Most studies used more than one method to assess nutritional status and the criteria used to define malnutrition was heterogeneous (table 2.1). The methods used to identify undernutrition were; weight measures in 23/46 studies, BMI in 16/46 and, MUAC and TSF in 11/46, whilst the methods used to identify overnutrition were measures of weight in 7/46 studies and BMI in 18/46. Studies investigating overnutrition did not used MUAC or TSF measurements. Finally, 13/46 studies explored linear growth expressed as height and/or height for age (HFA).

Table 2-1 Prevalence of undernutrition by type of measurement

Stage of disease	BMI, WFH		MUAC, TSF	
	Range (%)	Median (%)	Range (%)	Median (%)
At diagnosis	0-50	13	0-73	29·5
On treatment	0-50	16	0-34	30
End of treatment	0-46	20	0-20	19
Overall	0-50	15	0-73	29·5

BMI: body mass index; WFH: weight for height; MUAC: mid-upper arm circumference; TSF: triceps skinfold thickness.

2.1.3.3 Prevalence of undernutrition and overnutrition

Studies identified the prevalence of undernutrition employing BMI, weight loss, MUAC and TSF (tables 2.2, 2.3, 2.4 and 2.5). For comparative reasons, the prevalence of undernutrition was separated into two groups; those identified using weight loss, weight SD and BMI and those using MUAC and TSF. At diagnosis and during treatment, the prevalence of undernutrition (median %) was higher in studies employing MUAC and TSF than those using BMI and weight, whilst at the end of therapy this difference was not seen (table 2.5). Finally, the prevalence of overnutrition was established by selecting studies reporting BMI values (table 2.6).

Table 2-2 Studies reporting prevalence of undernutrition and overnutrition in paediatric cancer- Heamatological malignancies.

Author	Quality	Patients Dx	N, age (years)	Method Time of measurement	Method		Results		
					Design	Parameters	At diagnosis	During treatment	After treatment
(González et al. 2004) Cuba	Weak	ALL	N=49 Mean (6) Range (1-15)y	M: one observer T: Diagnosis After intensive phase End of Therapy	PC	Height, weight, BMI, TSF < 3rd centile of Cuban standards	0%	NR	NR
(Antillon et al. 2008) Guatemala	Moderate	ALL	N=164 Mean (±SD) 9·91 (±4.10)y	M: One observer T: Diagnosis 3 months 6 months	PC	Adequate: TSF, MUAC >10th percentile Depleted: TSF, MUAC 5-10th percentile Severely Depleted MUAC, TSF <5th percentile	54% Moderately &/or severely depleted	34% Moderately &/or severely depleted	19% Moderately &/or severely depleted
(Jaime-Pérez et al. 2008) Northern Mexico	Weak	ALL	N=102, Median (range) 6 (2-15)y	M: not mentioned T: Diagnosis On repeated occasions for 5 years	PC	BMI: percentiles of US standards Underweight <10th Normal weight 10-85th Risk of overweight 85-95th Overweight	Underweight: 12% (12/102) Normal weightt: 65% (66/102) Risk of overweight: 9% (9/102)	NR	NR

						>95th	Overwt: 15%(15/102)		
(Gofman and Ducore 2009) California, USA	Strong	ALL NHL	N=95 ≤18 years Median (range) ?	M: medical records T: Diagnosis, end of therapy & 2 years after treatment	RC	BMI: of US standards Overweight: Z-scores 1-1·65SD Percentiles 85th - 95th Obesity Z-scores >1·65 Percentile >95th	Overweight 6% Obese 2%	Overweight 20% Obese 12%	Overweight 16% Obese 24%
(Baillargeon et al. 2007) Texas, USA	Moderate	B-precursor ALL	N=307 Median (range) NR (2-18)y	M: medical records T: Diagnosis to 30 months and follow up every 6 months	RC	Overweight BMI 85-95th percentile Obese >95th percentile	Overweight: 10·8% Obese: 15·3%	NA	NA
(Baillargeon et al. 2005) Texas, USA	Weak	B-precursor ALL	N=141 Median (range) NR (2-18)y	M: medical records T: Diagnosis to 30 months and follow up every 6 months Hispanics only	RC	Overweight 85-95th percentile Obese >95th percentile	Normal weight: 70% (99/141) Overweight: 13% (18/141) Obese: 17% (24/141)	24 months Normal weight: 71.9%(77/107) Overweight: 12.1% (13/107) Obese: 16% (17/107)	30 months Normal weight: 71% (70/98) Overweight:13% (13/98) Obese: 15% (15/98)
(Odame et al. 1994) Scotland, UK	Weak	Cohort: ALL Controls: Wilm's tumour, non-Hodgkin's	N=40 cohort N=18 controls	M: medical records T: Diagnosis 2 years and 4	RC	BMI z score Overnutrition criteria according to French	Obesity in ALL cohort: Boys: 5% Girls: 5%	Obesity in ALL cohort: Boys: 26% Girls: 43%	Obesity in ALL cohort: Boys: 21% Girls: 57%

		lymphoma, rhabdomyosarcoma, Neuroblastoma, LCH & yolk sac tumour	Cohort: Mean boys: 3·4y girls 3·6y Controls: Mean boys 3·9y girls 3·7y	years		population standards (Rolland-Cachera et al)	Obesity in Controls: Boys:0% Girls:10%	Obesity in Controls: Boys: 0% Girls: 20%	Obesity in Controls: Boys: 25% Girls: 20%
(Delbecque-Boussard et al. 1997) France	Weak	ALL	N= 15 Mean (SD) 6·12(±3.2)y	M: NR T: day 1, 22, 36 & 71	PC	WFH according to charts of Sempe et al MUAC: NR TSF: NR	Undernourished 2/15 (13%)	Results were similar to diagnosis for day 22, 36 & 71	NA
(Bond et al. 1992) UK	Weak	ALL and Solid tumours Controls	N=26 Mean (±SD) 10·1 (±0.6)y N=26 Mean (±SD)10 (±0·6)y	M: NR T: 6 months from diagnosis and 1 week before CT	PC	WFH & HFA standards according to Waterlow et al TSF for FM not mentioned	NA	4/26 (15%) undernourished 1 (4%)ALL <90% HFA (stunted) 3 (11.5%) solid tumours <80% WFH (wasted)	NA
(Uderzo et al. 1996) Italy	Weak	Newly diagnosed Leukaemia	Cohort N=173 Median (range) 4 (0·2-15)y Control N=307 Median (range)	M: two observers T: at diagnosis	C	Data (height, weight, WFH, MUAC & TSF) compared with the national center for health statistics in the USA	Cohort v controls (p>0·05) Cohort 7% had WFH <90% Controls 8·5% (26) had WFH <90%	NA	NA

			7(5-14)y				MAC Cohort 2% <90% Controls 2% <90% TSF Cohort 18% Controls 11%		
(Reilly et al. 1999) UK	Strong	Standard Risk ALL	N=1013 Median (range) Boys: 4·4 (0·4-14·9)y Girls: 4·4 (0·2-14·9)y	M: Medical records T: at diagnosis	RC	BMI SD UK population reference data Undernutrition (-2 SD) Overnutrition(+2 SD)	Undernutrition: Boys: 8% Girls: 7% Overnutrition: Boys: 3% Girls:3%	NA	NA
(Viana et al. 1994) Brazil	Moderate	ALL	N= 128 Median (range) 5·8 (0·7-14·7)y	M: NR T:at diagnosis	PC	WHO standards WFA, WFH. HFA Undernutrition (Z score -2 SD)	Undernutrition: WFA: 21% HFA: 17%	NA	NA
(Argüelles et al. 2000) Spain	Weak	ALL	N=26 Mean 5·12y Range 1·66-10y	M: NR T: Diagnosis, 6, 12, 18, 24, 30 & 36 months	PC	Normal BMI & Ht +1 SD to -1 SD according to Spanish standards	Mean (±SEM) Ht SD 0.55 (±0·19) BMI NR Prevalence NR	Mean (±SEM) Ht SD At 6 months 0·4(±0·19) At 12 months 0·71(±0·22) At 18 months 0·78(±0·2) At 24 months 0·97(±0·21) At 30 months	Mean (±SEM) Ht SD 1·03 (±0·2)

								1·02(±0·22) At 36 months 1·03(±0·20)	
(Murphy et al. 2006) UK	Weak	ALL	N=29 ALL group Mean(±SD) age 9·6(±1·8)y Healthy control group Mean(±SD) 9·6 ± 2·0y	M: 1 observer T: off treatment (range 1-2y) Diagnosis to 5 years	PC	NR	NR	NR	% FM and FM were higher in ALL survivors FFM Index was significantly different between ALL and the control group No sig differences between Pred and Dexa group but the latter had a mean FM 6%higher
(Halton et al. 1998) Canada	Weak	ALL	Study I: N= 116 Study II: no relevant Study III: N= 19	M: NR T: Study I Diagnosis 12 months intervals during treatment 12 months after completion of therapy Study II: not relevant	PC	Not mentioned All measurements are compared with baseline	**Study I** **Weight:** Standard Risk: Mean SD:0·00 Males Mean SD: 0·36 Females Mean SD: 0·15 High risk: Mean SD: 0·2 **Study III** **% fat mass**	**Study I** **Weight** Reduction in weight velocity during 1st year and a disproportionate ↑ in weight thereafter At 24 months Standard Risk: Mean SD:0·16	Catch up growth **Study I** **Weight:** Obesity tendency Standard Risk: Mean SD:0·47 Males Mean SD: 0·38 Females mean SD: 0·75 High risk: Mean SD: 0·35

				Study III: Diagnosis and 6 months intervals			21·5±6·1	Males Mean SD: 0·55 Females mean SD: 0·76 High risk: Mean SD: 0·4 **Study III** **Fat mass %** Increase to 25±8·9 at 12 months **Muscle mass** Reduced to 59·8±3·5 at 12 months	**Study III** **% fat mass** 27·7±9·9
(Ahmed et al. 1997) UK	Moderate	ALL	N=31 Median (range) age at diagnosis 4·4(1·1-14)y	M: one observer T: monthly measurements (range 3-24months)	PC	No outcomes measures	NA	Median Wt SD ↑ sig over 1st months and by the end of CT Median BMI SD ↑ sig at 6 and 10 months	NA
(van der Sluis et al. 2002) Netherlands	Weak	ALL	N=61 Median (range) age 7·1(1·6-16·8)y	M: not mentioned T: At diagnosis, 32 weeks, 1, 2 and 3 y	PC	Ht and BMI compared with age and sex matched reference values from Netherlands Measurements compared to baseline	Ht: mean -0·19 SD BMI: -0·31SD **values are approximate-obtained from table with the use of a ruler*	**32 weeks** Height was decreased significantly to -0·75 SD BMI: sig increased to +0·65 SD **2 year** Ht: -0·75 SD BMI: +1·35 SD	1 y after cessation of therapy sig increase in ht Ht: -0·63SD BMI: +1·05 (decrease non-sig)

(Groot-Loonen et al. 1996) Netherlands	Weak	ALL	N=92 High risk n=15 Mean age 6·7y Range 1·1-15·9y Normal risk N=77 Mean age 5.2y Range0.6-15.5y	M: experienced staff T: diagnosis to 2 years (8-12 times per year)	PC	WFH Weight Dutch reference standards	NR	Patients treated with Dexa had higher WFH than those treated with Pred Weight did not differ between groups Weight did not sig differ in boys and girls	NA
(Koskelo et al. 1990) South Africa	Weak	Heamatological malignancy	Cohort: N=12 Mean 5·9y Range (1·8-13·6)y Controls: N=31 Mean: NR Range (3·6-13·9)y	M: one investigator T: at diagnosis, at 2-week interval up to 24 weeks	PC	Reference healthy controls Weight, height, MUAC & TSF	Cohort Median relative body weight (RBW) +2% in compare to controls	RBW increase to 8% Muscle wasting decrease by 27% average Increase of TSF	NA

Dx: Diagnosis; M: method; T: time of measurement; C: cohort study; PC: prospective cohort study; RC: retrospective cohort study; CS: cross-sectional study; NR: not reported and/or not clear; NA: non-applicable (i.e. not part of study aims); Measurements: BMI: body mass index; Ht: height; WFH: weight for height; WFA: weight for age; HFA: height for age; MUAC: middle upper arm circumference; TSF: triceps skinfold thickness; FM: fat mass; FFM: free fat mass; RBW: relative body weight; Diagnosis: ALL: acute lymphoblastic leukaemia; HM: Heamatological malignancies; ICCC-3: International classification of Childhood Cancer, Third edition; NHL: non-Hodgkin's lymphoma; Treatment: CT: chemotherapy; Dexa: Dexamethasone; Pred: Prednisolone; R: radiotherapy; ↓: decrease; ↑: increase; SD: standard deviation; y: years; sig: significant. Countries: UK: United Kingdom; USA: United States of America

Table 2-3 Studies reporting prevalence of undernutrition and overnutrition in paediatric cancer- Solid and brain tumours

Author	Quality	Patients Dx	N, age	Method & Time of measurements	Method		Results		
					Design	Parameters	At diagnosis	During treatment	After treatment
(Elhasid et al. 1999) Israel	Weak	Solid tumours	N= 50 Median (range) ? (0·17-17·5)y	M: NR T: Diagnosis & after each course of CT	PC	Weight; weight loss > 5%	weight loss %: NR	Weight loss %: NR	Weight loss %: NR
(Schiavetti et al. 2002) Italy	Weak	Solid tumours	N=36 On treatment group (n=19) mean(±SD) 9·7(±4·9)y Off treatment group (n=17) mean(±SD) 10·4(±4·6) y	M; One observer T: on treatment & off treatment (1-2 y)	PC	RBW% according to Italian obesity consensus conference: < 90% for underweight 90-110% for normal weight 110-120% overweight >120 obese BMI center for disease control recommendation: <5th underweight 5th-84th normal weight ≥overweight ≥95th obese	NA	Prevalence of underweight: On treatment group: %RBW: 26% BMI: 16% Prevalence of overweight and obese: On therapy group: %RBW: 40% BMI: 21% Off therapy: %RBW: 53% BMI: 35%	Off treatment group Underweight 0% Overweight and obese 35%
3. Bakish et al 2003	Strong	Newly diagnosed medulloblastoma	N= 112 Median	M: hospital records	RC	IBW determined using Tanner-	Median % of IBW at	46% of patients on	NA

Canada & UK		& supratentorial PNET	age (range) 6·1 (0·1-15·4)y	T: At diagnosis Before and after treatment (SG, CT, RT) Before, 1 & 3 months after dietetic intervention		Whitehouse growth charts Risk for undernutrition: weight < 90% of their IBW Significant weight loss/gain defined as change of > 5% Stable weight change < 5%	diagnosis 94% 31% at risk of undernutrition	CT lost > 5% TBW	
(Barbosa-Cortés et al. 2007) Mexico	Weak	Lymphoma and solid tumour	N=17 Mean (±SD) Lymphoma (n=8): 11·1 (±3·2)y Solid tumour (n=9): 9·5(±4·1)y	M: one observer T: after 1st CT course & 2 & 6 months into treatment	PC	To compare whether there is differences between lymphoma & solid tumour groups in body composition	4/17 (23·5%) children had a BMI Z-score <-2 SD		Body weight ↑significantly in the lymphoma group only
(Green et al. 2008) Canada	Weak	Stage IV NB	N=12 Mean (±SD) 3·8 (1.5)y Range 0·5* -18y	M: one observer T: Diagnosis (phase 1) After 2 courses of CT (phase 2) 2 or more weeks after surgical excision (phase 3)	PC	Undernutrition criteria: 2 or more: WFH <85% TSF <5th percentile	50% Undernourished	30% Undernourished	20% Undernourished

				End of therapy (final phase)					
(Oguz et al. 1999) Turkey	Weak	Cohort :Solid tumours Control: no sex and aged matched healthy children	N= 62 (cohort) Mean (±SD) age: 6·5 (±3·7)y Range: (0·08-13)y N= 31 (healthy control) Mean (±SD) age: 5·7 (±4·7)y Range: 0·25-15)y	M: one observer T: at diagnosis	PC	WFH (WHO standards) MUAC & TSF (Frisancho 1994 standards)	27% Undernourished (MUAC & TSF)	NA	NA
(Wessels et al. 1999) South Africa	Weak	Wilms' tumour	N=59 Undernourished group Median (range) 3·0(0·5-8·2)y Well-nourished group Median (range) 2·9	M: charts review T: at diagnosis, end of therapy	RC	WFA & WFH WHO standards Undernutrition <3rd centile or <90th centile	35% were undernourished		NR

			(0·5-11·6)y*						
(Müller et al. 2001) Germany	Strong	Craniopharyngioma	N=185 Median (range) 8.6 (0.1-18)y	M: medical records T: At diagnosis up to a median (range) 6·6 (0·1-34)y	RC	Rolland Cachera et al 1991 BMI: Obesity>3SD Overweight 2-3 SD Normal weight <2SD	NR	Normal weight: 43% Overweight: 13% Obesity 44%	NR
(Müller et al. 2011) Germany	Weak	Craniopharyngioma	N=120 Median(range) 10(1·2 - 18)y	M: not mentioned T: At diagnosis, at 36 months	PC	BMI SD to measure level of obesity	The degree of obesity was similar in patients with different grades of hypothalamic involvement Prevalence: NR	NA	Surgical hypothalamic lesions of anterior and posterior hypothalamic areas are associated with higher ↑ in Mean BMI SD +3·22 SD in comparison to patients with no lesions: Mean BMI SD: +0·45 and 1 anterior lesion Mean BMI SD: +0·74 Prevalence NR
10. Israels et al.	Weak	Wilm's tumour	N= 19 Malawi	M: NR T: At diagnosis	CS	HANES growth curve (1978)	Malawi: Z-score	NA	NA

(2010)(Israels et al. 2010) Malawi & UK	N=11 Mean age (±SD) 4·5±2·6y United Kingdom N=8 Mean age (±SD) 4·6±2·1y	corrected WFH: -2·3±1·3 UK: Z-score corrected WFH: 0·4±0·8

Dx: Diagnosis; M: method; T: time of measurement; PC: prospective cohort study; RC: retrospective cohort study; CS: cross-sectional study; NR: not reported and/or not clear; NA: not applicable (i.e. not part of study aims); Measurements: BMI: body mass index; WFH:weight for height; WFA: weight for age; HFA: height for age; MUAC: midd upper arm circumference; TSF: triceps skinfold thickness; FM: fat mass; FFM: free fat mass; RBW:relative body weight; Diagnosis: ALL: acute lymphoblastic leukaemia; HM: Haematological malignancies; ICCC-3: International classification of Childhood Cancer, Third edition; NB: neuroblastoma; PNET: primitive neuroectodermal tumor; Treatment: CT: chemotherapy; RT: radiotherapy; SG: surgery; ↓: decrease; ↑: increase; SD: standard deviation; *values converted from months to years. Countries: UK: United Kingdom.

Table 2-4 Studies reporting prevalence of undernutrition and overnutrition in paediatric cancer- ICCC-3 and other malignancies

Author	Quality	Patients Dx	N, age (years)	Method & Time of measurement	Method	Results			
					Design	Parameters	At diagnosis	During treatment	After treatment
(Sala et al. 2008) Guatemala	Strong	ICCC-cancer	N=472 Median (range) NR	M: one observer T: 48 hours maximum into treatment	C	TSF & MUAC Adequately nourished: >10th Depleted: 5th-10th Severely depleted: <5th MUAC & TSF (Frisancho 1981)	57% (271/472) undernourished 8% (41/472) severely undernourished Solid tumours: 57% (112/237) undernourished 10% (25/237) severely undernourished ALL 48% (97/202) undernourished 6% (13/202) severely undernourished Other leukaemias + MDS 64% (21/33) undernourished 9% (3/33) severely	NA	NA

							undernourished		
(Sala et al. 2012) Guatemala, El Salvador, Nicaragua, Dominican Republic, Panama, Honduras, Costa Rica	Strong	ICCC-3 cancer	N= 1787 Median (IQR) age 6·8y (3·8-10·6)y	M: one to two observers in each country T: Diagnosis	PC	BMI (WHO) < 15th centile undernourished: <5th centile severely undernourished MUAC & TSF (Frisancho 1981): 5-10th centile undernourished: <5th severely depleted	BMI: undernourished: 28% <5th severely undernourished 20% MUAC & TSF: undernourished: 18% severely depleted:45%	NA	NA
(Pietsch & Ford 2000) USA	Moderate	Cancer diagnosis	N= 133 Median age NR	M: medical records T: at diagnosis	RC	Undernutrition defined: WFH <50% percentile WFH <20% percentile WFH <80% of the median BMI <16 BMI z-score <-2·0 SD Reference for children BMI Z scores ≥5y (Rosner et al 1998) Z-scores for	Prevalence of undernutrition: WFH <50th: 38/81 (47%) WFH<20th : 12/81 (15%) Median WFH <80%: 1/81 (1%) WFH Z score: -2·00 SD 1/81 (1%) BMI <16 41/127 (32%)	NA	NA

						WFH, WFA & HFA in children ≤10y NCHS growth curves	BMI Z score - 2·00 SD 2/78 (3%)		
(Smith et al. 1991) UK	Moderate	Newly diagnosed malignancy	N=100 Median (range) 5(0·3-16·5)y	M: one observer T: diagnosis, every month *For how long?*	PC	Undernutrition defined as HFA -2 SD (WHO) TSF -2 SD WFH 80% of median MUAC 5th centile	HFA & WFH normal MUAC & TSF significantly < than reference & controls 20% had MUAC <5th centile 23% had TSF >2 SD below the mean	NR	NR
(Collins et al. 2010, Barr et al. 2011) Canada	Strong	Haematological malignancy and extracranial solid tumour	N=99 Median (range) 7·2 (2·1-15·2)y*	M: Registered dietitians T at diagnosis	C	BMI SD USA population reference data MUAC and TSF Frisancho Undernutrition <5th centile Risk of overweight 85th-95th Overweight >95th	Undernourished: BMI 9% MUAC 5% TSF 10% Risk of overweight BMI 18% MUAC 7% TSF 6% Overweight: BMI: 7% MUAC: 4% TSF:9%	NA	NA
(Pedrosa et al. 2000)	Moderate	Haematological malignancies and	N=443 Median	M:patient's records	RC	WHO standards WFA, WFH,	Undernutrition: WFA: 23·5%	NA	NA

Brazil		solid tumour	(range) 4·9 (0-17·8)y	T: at diagnosis, 1 year		HFA Undernutrition Z-scores -2 Outcome: survival at 1 year	WFH: 16% HFA: 23%		
(Skolin et al. 1997) Sweden	Weak	Leukaemia & solid tumours	N=14 Median (range) 10·0 (5·0-16·0) y	M: medical records (*only for weight)* T: Admission, 1 week, 6 weeks and 3 months after diagnosis	RC	Weight compared with NCHS growth curves	On admission Weight SD -0·09	Weight reduction: At 1 week: 0·19 SD At 6 weeks 0·10 SD At 3 months 0·37 SD All measurements compared to admission	NA
(Murphy et al. 2010) Australia	Weak	Cohort (malignancy) Control (healthy children)	N= 48 (control and cohort)	M: not mentioned T: between 1·2 and 2 y after diagnosis	CS	Comparison of height, weight and BMI between controls and cohort National standards		No sig difference in height, weight and BMI between cohort and controls	
(Zimmermann et al. 2013)	Strong	ICCC-3 cancer	N= 327	M: medical records T: from diagnosis until the end of therapy,	RC	Reference values: <4 y: WHO >4 y: KiGGS Undernutrition defined as:	Undernutrition BMI or weight loss: Overall: 6% Non-	Undernutrition: BMI or weight loss: ALL 44 (46%) AML 15 (58%) NHL 13 (46%)	NR

	relapse, death or continuation of treatment in a non-participating hospital. Intervals between measurement between 7-90 days	BMI Z-score -2 Weight loss > 10%	medulloblastoma CNS: 19% Osteosarcoma, medulloblastoma and neuroblastoma: 13%	HL 6 (24%) Medulloblastoma 15 (94%) Other CNS tumor 10 (39%) Rhabdomyosarcoma 8 (42%) Ewing sarcoma 8 (50%) Osteosarcoma 11 (73%) Nephroblastoma 8 (44%) Neuroblastoma 8 (50%) Other 9 (33%)

D: Diagnosis; M: method; T: time of measurement; C: cohort study; PC: prospective cohort study; RC: retrospective cohort study; CS: cross-sectional; NR: not mentioned and/or not clear; NA: non-applicable (i.e. not part of study aims); Measurements: BMI: body mass index; WFH:weight for height; WFA: weight for age; HFA: height for age; MUAC: midd upper arm circumference; TSF: triceps skinfold thickness; FM: fat mass; FFM: free fat mass; RBW:relative body weight; Diagnosis: ALL: acute lymphoblastic leukaemia; MDS: myelodysplastic syndrome; HM: Haematological malignancies; ICCC-3: International classification of Childhood Cancer, Third edition; Treatment: CT: chemotherapy; RT: radiotherapy; SG: surgery; ↓: decrease; ↑: increase; SD: standard deviation: y: years; sig: significance; Countries: UK: United Kingdom; USA: United States of America; *data converted from months to years.

Table 2-5 Studies reporting the prevalence of undernutrition (from all measurement types) categorised by stage of treatment and diagnostic criteria

	Total number of studies (n)	At diagnosis			During treatment			End of treatment		
		n	Range %	Median %	n	Range %	Median %	n	Range %	Median %
Haematological malignancies	8	6	0-54	13	4	0-34	14	2	0-19	9.5
Solid tumours	7	4	23.5-50	29.5	2	16-30	23	3	20-46	35
ICCC-3 + other cancers	5	5	3-65	20	1	50	50	none	none	none
Overall	17*	14*	0-65	23.5	6*	0-50	16	5	0-46	20

*some studies report prevalence for more than one category

Table 2-6 Prevalence of undernutrition by type of measurement: prevalence of undernutrition obtained by BMI and weight measurements compared to those obtained by arm anthropometry

	Overall		At diagnosis		On treatment		End of therapy	
	BMI (n=16) WT (n=24)	MUAC,TSF (n=11)	BMI (n=10) WT (n=9)	MUAC,TSF (n=7)	BMI (n=2) WT (n=2)	MUAC,TSF (n=1)	BMI (n=3) WT (n=1)	MUAC,TSF (n=1)
Median %	15	30	16	40	15	30	20	19
Range %	0-50	0-73	0-50	0-73	0-50	0-34	0-46	0-20

% percentage; n: number of studies; BMI: body mass index; WT: weight; MUAC: middle upper arm circumference; TSF: triceps skinfold

Table 2-7 Studies reporting prevalence of overnutrition defined by BMI and categorised by stage of disease and diagnostic criteria

Diagnostic Category	Total number of studies (n)	At diagnosis			During treatment			End of treatment		
		n	Range %	Median %	n	Range %	Median %	n	Range %	Median %
Haematological malignancies	5	5	8-30	15	3	28-69	40	3	28-78	40
Solid tumours	1	0	none	none	1	21	21	1	35	35
Brain tumours	1	1	NR	NR	1	57	57	1	NR	NR
ICCC-3 + other cancers	1	1	4-9	7	none	none	none	none	none	none
Overall	8	6	8-30	15	5	21-69	30	5	28-78	38

BMI: Body mass index; ICCC-3: International Classification of Childhood Cancer-3rd edition; NR: not reported.

2.1.3.4 Linear growth

Thirteen out of 46 studies explored growth defined as height and HFA (table 3.8). Standard measures of growth were defined in 7/13 studies and 6/13 either compared mean height (SD) at different stages of the disease with baseline or, described a height pattern throughout the course of treatment. At diagnosis (7/13), five studies reported a normal HFA in comparison to the national average but, two reported poor linear growth in 17% (Viana et al. 1994) and 23% (Pedrosa et al. 2000) children. 7/13 studies reported height SD changes during treatment. Of these, three studies reported normal linear growth; however, four reported a significant decrease in mean height SD from diagnosis or, values significantly lower than the HFA national averages. Finally, 5/13 studies reported height changes at the end of therapy. Of these, one reported normal heights in compared to the national standards, whilst four reported some catch up growth; however, mean height SD remained lower than the national average.

2.1.3.5 Associations between malnutrition and outcome

Nine out of 46 studies were found (see table 3.9) to explore associations between undernutrition and clinical outcome in children with cancer; two of these were preliminary data (Sala et al. 2008, Antillon et al. 2008) of a later study (Sala et al. 2012). The outcomes measured were; relapse, mortality, abandonment of therapy, treatment toxicity, event free survival rates (EFS), type of tumour and age at diagnosis**.** The performance of a meta-analysis was impossible due to the heterogeneity of the outcomes measured, the variability found in the anthropometrical measurements and the heterogeneous populations. Therefore, evidence was described narratively. Three studies (Reilly et al. 1994, Sala et al. 2012, Weir et al. 1998) reported data on relapse events and of these, two did not find that undernutrition (established with BMI SD) at diagnosis had any significant influence on either time of relapse (Weir et al. 1998) or relapse rates (Sala et al. 2012); whilst one reported that children who were undernourished (established using WFH SD measurements) at diagnosis were more likely to relapse (Reilly et al. 1994). Two out 9 studies undertaken in developing countries investigated the degree of undernutrition and mortality rates establishing that the more undernourished the

children were the higher the mortality rates (Sala et al. 2012, Mejía-Aranguré et al. 1999). One out 7 studies established that undernourished children were more likely to abandon therapy and this was more noticeable in children with solid tumours and heamatological malignancies (Sala et al. 2012). Another study found that 2-year EFS was significantly different between well-nourished and undernourished children, whereby 65% and 48% survived respectively (Sala et al. 2012). Additionally, one study explored factors associated with undernutrition (Zimmermann et al. 2013). This study found that children diagnosed with medulloblastoma, who were older than 10 years of age at the time of diagnosis and had severe induced side effects (vomiting), were at higher risk of becoming undernourished (Zimmermann et al. 2013). Finally, one cross-sectional study established that undernourished children from Malawi diagnosed with large Wilms tumours exhibited lower chemotherapy (vincristine) clearance than their well-nourished counterparts from the UK (Israels et al. 2010).

One study investigated whether obesity in children diagnosed with ALL was associated with survival and EFS, but no significant associations were established (Baillargeon et al. 2006).

Table 2-8 Studies reporting linear growth (height and height for age)

Author	Quality	Patients Dx	N, age (years)	Method & time of measurement	Method		Results		
					Design	Parameters	At diagnosis	During treatment	After treatment
(Argüelles et al. 2000) Spain	Weak	ALL (HM)	N=26 Mean 5·12y Range 1·66-10y	M: NR T: Diagnosis, 6, 12, 18, 24, 30 & 36 months	PC	Normal BMI & Height ±1 SD according to Spanish standards	Mean (±SEM) Height SD 0·55 (±0.19) BMI NR	Mean (±SEM) Height SD At 6 months 0·4(±0·19) At 12 months 0·71(±0·22) At 18 months 0·78(±0·2) At 24 months 0·97(±0·21) At 30 months 1·02(±0·22) At 36 months 1·03(±0·20)	Mean (±SEM) Height SD 1·03 (±0·2)
(Murphy et al. 2006) UK	Weak	ALL (HM)	N=29 ALL group Mean(±SD) age 9.6(±1.8)y Healthy control group Mean (±SD) 9.6 ± 2.0y	M: one observer T: off treatment (range 1-2y) Diagnosis to 5 y	PC	NR	NR	NR	Females with ALL had a tendency for ↓height & ↑FM (FM 60%higher than control group)
(Bond et al. 1992) UK	Weak	ALL (HM) & Solid tumours	Cohort N=26 Mean (±SD) 10·1 (±0·6)y	M: NR T: 6 months from diagnosis and 1 week	PC	WFH & HFA according to Waterlow et al	NA	1 (4%)ALL <90% HFA (stunted)	NA

		Controls	Controls N=26 Mean (±SD) 10 (±0·6)y	before CT		TSF for FM not mentioned		ALL group tended to be shorter	
(Halton et al. 1998) Canada	Weak	ALL (HM)	Study I: N= 116 Study II: no relevant Study III: N= 19 Median (range) NR	M: not mentioned T: Study I Diagnosis 12 months intervals during treatment 12 months after completion of therapy Study II: not relevant Study III: Diagnosis and 6 months intervals	PC	Standard measures not mentioned All measurements are compared with baseline	**Study I** **Height:** Standard risk: Mean SD -0·03 Males Mean SD -0·18 Females Mean SD 0·3 High Risk: Mean SD 0·28 **Weight:** Standard Risk: Mean SD:0·0 Males Mean SD: 0·36 Females mean SD: 0·15 High risk: Mean SD: 0·2 **Study III** **% fat mass** 21·5±6·1 **Protein (g/l)** 65·9±11·9	**Study I** **Height:** Decrease in mean height SD during treatment (12 and 24 months) In the standard and high risk group At 24 months: **Weight** Reduction in weight velocity during 1st year and a disproportionate increase in weight thereafter **Study III** **Fat mass %** Increase to 25·0±8·9 at 12 months **Muscle mass** Reduced to 59·8±3·5 at 12 months	Catch up growth **Study I** **Height:** Standard risk: Mean SD -0·58 Males Mean SD -0·31 Females Mean SD -0·15 High Risk: Mean SD -0·46 **Weight:** Obesity tendency **Study III** **% fat mass** 27·7±9·9 **Protein (g/l)** 57·9±2·4 (sig reduction from diagnosis)

(Smith et al. 1991) UK	Moderate	Newly diagnosed malignancy (ICCC-3)	N=100 Median (range age 5 (0·3-16·5)y	M: one observer T: diagnosis, every month *For how long?*	PC	Undernutrition defined as HFA -2 SD (WHO)	HFA & WFH normal	NR	NR
(Uderzo et al. 1996) Italy	Weak	ALL (HM)	Cohort N=173 Median (range) age 4 (0·16-15) y Control N=307 Median (range) age 7 (5-14)y	M: two observers T: at diagnosis	PC	Data (height, weight, WFH, MUAC & TSF) compared with the national center for health statistics (NCHS) in the USA	Height measurements NR	NA	NA
(Pietsch and Ford 2000) USA	Moderate	Cancer diagnosis (ICCC-3 & other)	N= 133 Median age NR	M: medical records T: at diagnosis	RC	Z-scores for WFH, WFA & HFA in children ≤10y NCHS growth curves	HFA NR	NA	NA
(Ahmed et al. 1997) UK	Moderate	ALL (HM)	N=31 Median (range) age at diagnosis 4·4 (1·1-14)y	M: one observer T: monthly measurements (range 3-24months)	PC	NR	NA	Height SD is lowest at 6 months after CT & remains significantly depressed until the end of the 1st year Median Weight SD ↑ sig over 1st months & by the end of CT	NA

								Median BMI SD ↑ significantly at 6 & 10 months	
(van der Sluis et al. 2002) Netherlands	Weak	ALL (HM)	N=61 Median (range) age 7·1(1·6-16·8) y	M: NR T: At diagnosis, 32 weeks, 1, 2 and 3 years after diagnosis	PC	Height and BMI compared with age & sex matched reference values from Netherlands Measurements compared to baseline	Height: mean -0·19 SD BMI: -0·31SD **values are approximate-obtained from table*	32 weeks Height ↓ significantly to -0·75 SD BMI: sig ↑ to +0·65 SD 2 year Ht: -0·75 SD BMI: +1·35 SD	1 year after cessation of therapy: sig ↑ in height Height: -0·63SD BMI: +1·05 (↓ non-significant)
(Tamminga et al. 1992) Netherlands	Weak	ALL (HM)	N=53 Group I: Median (range) age 4·5(2·4-10·4)y Group II: Median (range) age 5·3 (1·6-14·0)y Group III: Median (range) age 5·6 (2.4-12.2)y Group IV: Median (range) 8·3 (4·8-13·4)y	M: NR T: every 3 months for 2 years Group I: ALL High Risk Group II: ALL irradiated Group III: ALL not irradiated Group IV: ALL after therapy	PC	Height, weight, WFH, MUAC, sitting height, arm span and head circumference	Height SD: I: 1·3 SD II: -0·1 III: +0·05 IV: +1·3	Height retardation was maximal in the first 6 months after diagnosis No sig difference between groups during treatment Sig ↑ in height in groups II & III (when NR)	NA
(Pedrosa et al. 2000) Brazil	Moderate	HM and solid tumours	N=443 Median (range) age 4·9 (0-17·8)y	M:patient's records T: at diagnosis, 1 year	RC	WHO standards WFA, WFH, HFA Undernutrition Z-scores -2	Undernutrition: WFA: 23·5% WFH: 16% HFA: 23%	No sig difference in survival rates between well-nourished and undernourished	

						Outcome: survival at 1 year		children	
(Caruso Nicoletti et al. 1993) Italy	Weak	ALL CR Non-CR	N= 50 Median (range) age 5 (1·6-11·9)	M: one observer T: at diagnosis, every 3 months during treatment and every 6 months after treatment for 5 years.	PC	Height SD and height velocity Tanner's data	Normal height at diagnosis	Both groups exhibited a ↓ in growth velocity during the first 6 months Growth deprivation was more severe in the CR group During the 2nd year growth velocity returned to normal	Catch up growth until almost normal for both groups
(Viana et al. 1994) Brazil	Moderate	ALL	N=128 Median (range) age 5·8 (0·75-14·75)y	M: NR T: at diagnosis	PC	WHO standards	Reduced HFA: 23%	NA	NA

D: diagnosis; M: method; T: time of measurement; PC: prospective cohort study; RC: retrospective cohort study; NR: not reported and/or not clear; NA: not applicable (i.e. not part of study aims); Measurements: BMI: body mass index; WFH: weight for height; WFA: weight for age; HFA: height for age; MUAC: midd-upper arm circumference; TSF: triceps skinfold thickness; FM: fat mass; FFM: free fat mass; Diagnosis: ALL: acute lymphoblastic leukaemia; HM: Haematological malignancies; ICCC-3: International classification of Childhood Cancer, Third edition; Treatment: CT: chemotherapy; R: radiotherapy; CR: cranial radiotherapy; ↓: decrease; ↑: increase; SD: standard deviation; y: years; sig: significant; Countries: United Kingdom; USA: United States of America.

Table 2-9 Associations between malnutrition and outcomes

Author	Quality	Patients Dx	N, age	Time of measurement/method	Method			Results
					Design	Variables	Outcomes	
(Sala et al. 2012) Guatemala, El Salvador, Nicaragua, Dominican Republic, Panama, Honduras, Costa Rica	Strong	Childhood cancer (ICCC-3 criteria)	N= 1787 Median (IQR) age 6.8y (3.8-10.6)	T: Diagnosis M: one to two observers in each country	PC	Nutritional status assessed with BMI, MUAC & TSF	Relapse Abandonment of treatment EFS CT interruptions	No association between nutritional status and relapse Undernourished children abandoned treatment more often ($p<0.05$) Undernutrition was associated with reduced EFS and ↑ CT interruption.
(Weir et al. 1998) UK	Strong	Standard risk ALL	N=1025	M: medical records T: at diagnosis	RC	BMI SD UK national standards Undernutrition (-2 SD) Overnutrition(2 SD)	Relapse rates	BMI SD at diagnosis did not influence statistically time of relapse
(Reilly et al. 1994) UK	Moderate	Standard risk ALL	N=78	M: NR T: at diagnosis	PC	WFH SD UK population reference data Undernutrition	Relapse rates	Children with the lowest WFH SD score at diagnosis were at higher risk of early

						(-2 SD) Overnutrition(2 SD)		relapse than their well-nourished counterparts
(Viana et al. 1994, Pedrosa et al. 2000) Brazil	Moderate	Heamatological malignancies and solid tumours	N=443 Median (range) 4·9 (0-17·8)y	M:patient's records T: at diagnosis, 1 year	RC	WHO standards WFA, WFH, HFA Undernutrition Z-scores -2	Survival at 1 year	No sig difference in survival rates between well-nourished and undernourished children
(Viana et al. 1994) Brazil	Moderate	ALL	N= 128 Median (range) age 5·83 (0·75-14·75)y	M: NR T:at diagnosis	PC	WHO standards WFA, WFH. HFA Undernutrition defined as Z score -2 SD	Relapse, remission, deaths, second tumour, EFS	Undernutrition was an adverse factor affecting duration of complete remission
(Wessels et al. 1999) South Africa	Weak	Wilms' tumour	N=59 Undernourished group Median(range) age 3 (0·5-8·25)y Well-nourished group Median (range) age 2.91 (0·5-11·58)y	M: charts review T: at diagnosis, end of therapy	RC	WFA & WFH WHO standards Undernutrition <3rd centile or <90%	Survival	No difference in survival between the two groups 74% of undernourished children survived in compare to 56% of the well-nourished group
(Mejía-Arangure et al. 1997) Mexico	Strong	ALL High Risk (HR) Low risk (LR)	N=105 Median NR	M: four observers T: at diagnosis, 2 and 3 months	PC	WFH Waterlow classification MUAC Frisancho standards Undernutrition	Risk of mortality	Undernutrition was associated with higher risk of mortality

						defined as ≥10% loss of MUAC		
(Israels et al. 2010) Malawi, UK	Weak	Wilm's tumour	N=19 Malawi (n=11) UK (n=8)	M: NR T: At diagnosis	CS	Malawi: Z-score corrected WFH: -2·3±1·3 UK: Z-score corrected WFH: 0·4±0·8	Vincristine clearance	Reduce Vincristine clearance was associated with undernutrition.
(Zimmermann et al. 2013)	Strong	ICCC-3	N=327	M: medical records T: from diagnosis until the end of therapy, relapse, death or continuation of treatment in a non-participating hospital. Intervals between measurement between 7-90 days	RC	Undernutrition assessed with BMI Z-score (-2) and weight loss % (10%)	Patient's related factors: age, gender and country of origin Disease factors: type of cancer, date of diagnosis, metastasis or CNS involvement Treatment related factors: emetogenicity	Medulloblastoma, age >10 y and high emetogenicity was sig. associated with ↑ risk of undernutrition.

Dx: Diagnosis; M: method; T: time of measurement; C: cohort study; PC: prospective cohort study; RC: retrospective cohort study; CS: cross-sectional study; NR: not reported and/or not clear; NA: non-applicable; Measurements: BMI: body mass index; Ht: height; WFH: weight for height; WFA: weight for age; HFA: height for age; MUAC: middle upper arm circumference; TSF: triceps skinfold thickness; FM: fat mass; FFM: free fat mass; RBW: relative body weight; Diagnosis: ALL: acute lymphoblastic leukaemia; HM: Heamatological malignancies; ICCC-3: International classification of Childhood Cancer, Third edition; NHL: non-Hodgkin's lymphoma; Treatment: CT: chemotherapy; Dexa: Dexamethasone; Pred: Prednisolone; R: radiotherapy; ↓: decrease; ↑: increase; SD: standard deviation; y: years; sig: significant. EFS: event free survival; Countries: UK: United Kingdom; USA: United States of America

2.1.3.6 Quality of body of evidence

According to the assessment of quality of evidence by CASP methods, the evidence for the prevalence of malnutrition in childhood cancer was mainly of poor quality. The main issues identified with studies scored as "weak" were:

(i) It was difficult to establish the precision of the results due the absence of confidence intervals and relative risk (n=29).

(ii) Confounding factors were not taken into consideration in the design and in the analysis of the data (n=18)

(iii) Anthropometrical data was not compared to either national or international standards making it very difficult to identify malnutrition in this population (n=10)

(iv) Inappropriate use of statistical methods (n=7)

Despite this, all selected studies were considered to draw some general conclusions.

2.1.4 Discussion

The results from this systematic review of 46 studies show that, to date, there is insufficient robust evidence to accurately determine the prevalence of malnutrition in paediatric cancer worldwide, particularly from the developing world. Moreover, evidence was heterogeneous with regards to the anthropometric measurements, the criteria and the cut-off values used to assess nutritional status. These limitations meant that it was impossible to perform a meta-analysis of the associations between malnutrition and clinical outcomes.

2.1.4.1 Prevalence of undernutrition and overnutrition

Evidence of the prevalence of malnutrition at different stages of the disease was highly variable across all types of paediatric cancer and, as expected, differences were observed between the developed and developing world. Three quarters of studies were performed in the developed world, though most cancer diagnoses occur in the developing world (Pritchard-Jones et al. 2013). The majority of the studies considered malnutrition in ALL patients, and the data for malnutrition in either solid

or brain tumours are limited. Moreover, most research, until now, has focused on the prevalence of undernutrition, whilst overnutrition has largely been overlooked. Finally, most studies investigated the prevalence of malnutrition at diagnosis with very few exploring nutritional status during or at the end of therapy.

Children diagnosed with haematological malignancies, mainly ALL, had the lowest prevalence of undernutrition; consistent with previous reviews (Sala et al. 2004, Brouwer et al. 2007). Contrary to previous studies (Sala et al. 2004, Carter et al. 1983), this review suggests that the prevalence of undernutrition in this group, in both developed and developing countries, is still significant, especially at diagnosis (median 13%) and during treatment (median 14%). Of note, there was more variability in the prevalence of undernutrition in developing countries (range 0-54%; median 23%) than in developed countries (range 4-15%; median 10%). Undernutrition in children with solid tumours was highly prevalent from diagnosis until the completion of therapy. The data for this subgroup, although variable, appeared more consistent. Nevertheless, a few cautionary notes are needed; the small number of studies looking at these patients (Barbosa-Cortés et al. 2007, Green et al. 2008, Oguz et al. 1999, Schiavetti et al. 2002, Bakish et al. 2003), their small sample sizes (range; 12-112) and the considerable differences in disease severity of the subjects included in each study as well as the site of treatment (Barbosa-Cortés et al. 2007, Green et al. 2008).

The median prevalence of undernutrition for all paediatric cancers (ICCC-3) was 20% at diagnosis. Although these studies were of high-quality with large sample sizes, the prevalence of undernutrition was extremely variable ranging from 3-65% (Sala et al. 2012, Sala et al. 2008, Barr et al. 2011, Pietsch and Ford 2000, Smith et al. 1991, Zimmermann et al. 2013). This may be due to the following: (i) cancer encompasses many diseases (Steliarova-Foucher et al. 2005), all treated with different forms, combinations and intensities of treatment by different specialised physicians (Chan 2007, Tobias, J, & Hochhauser, D 2010); (ii) the sample sizes of each type of cancer varied between the different studies; (iii) different crietria were used to identify undernutrition, (iv) and the standard population against which the studies compared their measurements was not always specified.

Only three studies considering overnutrition in haematological malignancies met the eligibility criteria (Gofman and Ducore 2009, Baillargeon et al. 2005, Odame et al. 1994). Although, they all reported similar prevalence (median 15%) at diagnosis their findings during and at the end of treatment were highly variable. Nonetheless, there is a suggestion that the number of cases of overnutrition increases during treatment (median 40%); in line with two reviews (Reilly 2009a, Brouwer et al. 2007). The available data for solid and brain tumours was even scarcer; prevalence of obesity during treatment was 21% (solid tumours) (Schiavetti et al. 2002) and 57% in patients diagnosed with brain tumours, particularly craniopharyngioma (Müller et al. 2001). The high incidence of obesity in children diagnosed with and treated for craniopharyngioma has been extensively discussed in a review, where obesity is attributed to a disruption of the hypothalamic function (Iughetti and Bruzzi 2011). This disruption is most commonly caused by the tumour itself or by the treatment (surgery or cranial radiotherapy) and can lead to abnormalities in both the satiety and the hunger control mechanisms, which in turn causes a dysregulation in energy balance (Iughetti and Bruzzi 2011). Other factors include sedentary behaviour, such as reduced physical activity (Iughetti and Bruzzi 2011)**.** At the end of therapy, only one study reported a prevalence of obesity of 35.5% (Schiavetti et al. 2002). Finally, one study of children diagnosed with a paediatric cancer (ICCC-3) reported a prevalence of 7% at diagnosis (Barr et al. 2011). Thus, with the present evidence, it is not possible to accurately report the prevalence of overnutrition in the different subpopulations of paediatric cancer. Although, studies were variable in terms of their quality, mainly due to methodological issues, on a positive note the criterion used to define obesity was described in all the studies and the methods employed to identify obesity were consistent; namely BMI +2SD.

Undernutrition in paediatric cancer at the time of diagnosis was more prevalent in developing (median 23·5%) than in developed countries (median 13%) (figure 3.2). This disparity may be multifactorial as reduced health care resources might contribute to either late diagnosis or treatment inaccessibility (Pritchard-Jones et al. 2013, Sala et al. 2004). Overnutrition at diagnosis was also higher in the developing world (15%) than in the developed world (10%). However, only one study from the

developing world (Mexico) reported prevalence of overnutrition (Jaime-Pérez et al. 2008), which is unlikely to be representative of the whole developing world.

2.1.4.2 Linear Growth

Children with ALL tended to have normal HFA at diagnosis, but a significant reduction in height velocity occurred during treatment, which remained until the end of therapy. At this stage, there was some catch up growth, but the HFA remained lower than the population average (Murphy et al. 2006, Halton et al. 1998, Ahmed et al. 1997, van der Sluis et al. 2002, Caruso Nicoletti et al. 1993, Holm et al. 1994). There were only two studies investigating linear growth in children diagnosed with solid tumours (Bond et al. 1992, Murphy et al. 2006); one reported normal heights at diagnosis (Murphy et al. 2006) and the other one reported that children with ALL tended to be shorter than those diagnosed with solid tumours (Bond et al. 1992). Thus, more research is needed to investigate linear growth in children diagnosed with solid tumours.

2.1.4.3 Methods used to identify malnutrition

The methods used to identify undernutrition were heterogeneous, whilst studies were more consistent in their assessment of overnutrition. At present, there is not a "gold standard" anthropometric measurement that best assesses undernutrition in ill children (Anon 2006b), whereas the evidence for the use of BMI to diagnose obesity is more consistent (Reilly 2006). It has been suggested (Sala et al. 2004) that arm anthropometry should be incorporated in clinical practice as a routine measurement (Sala et al. 2004, Sala et al. 2012, Oguz et al. 1999, Barr et al. 2011). This systematic review showed that the prevalence of undernutrition was highly variable regardless of the method employed; however, the median prevalence of undernutrition assessed by arm anthropometry (MUAC and TSF) was consistently higher than that obtained from BMI, both at diagnosis and during treatment, but it was almost the same at the end of therapy. This was the case for all diagnostic categories. The discrepancy between BMI and arm anthropometry may reflect the limitations of BMI in our population, not seen as much in healthy children (Cole et al. 1995a, Cole et al. 2007). BMI does not account for changes in body composition, weight of tumour and oedema (Oguz et al. 1999, Pietsch and Ford 2000, Smith et al. 1991, Murphy et al.

2010). MUAC and TSF are less influenced by these complications, hence a better reflection of body composition (Roche et al. 1981). Thus, this review proposes the following (table 2.10).

Table 2-10 Research recommendations to consider for the future

Research recommendations to consider for the future:

- Different forms of cancers need to be thought of as different diseases requiring very different treatments, as such should be presented separately.
- Measurements need to be collected at different stages of the disease and treatment course.
- A standard criterion needs to be applied to the diagnosis of both under and overnutrition using standard cut off values.
- Nutritional status should be assessed by weight, height (and the calculation of BMI), MUAC and TSF, not only for research purposes but also in the clinical setting.
- The following details need to be clearly specified: the timings of when the measurements are taken (before the start of treatment, at diagnosis, or during the initiation of treatment), the socio-economic status, ethnicity, disease severity, treatment protocol and nutritional support.

2.1.4.4 Nutritional status as a prognostic factor for outcome

There is a paucity of evidence examining associations between malnutrition at diagnosis and clinical outcomes and the few published studies appeared inconclusive. Undernutrition in paediatric cancer may be associated with higher mortality (Sala et al. 2012, Mejía-Aranguré et al. 1999); however, these two studies were both undertaken in developing countries and there may have been other factors related to mortality, such as increased duration of treatment (Viana et al. 1994) and, also toxicity (Sala et al. 2012). These findings are supported by a recent review (Sala et al. 2004) and evidence from the 1980s (Donaldson et al. 1981, Lobato-Mendizábal et al. 1989, Rickard et al. 1983). Notably, two large studies included in this review (Sala et al. 2012, Weir et al. 1998) did not find associations between undernutrition and relapse. Obesity in children with ALL was not associated with a decrease in EFS in comparison to their well-nourished counterparts (Baillargeon et al. 2006). In contrast, a large study (n=4356; age: 2-22 years), not included in the current review, showed that obesity at diagnosis independently predicted the likelihood of relapse in preteenagers and adolescents with ALL (Butturini et al. 2007). This effect was independent of disease severity, chemotherapy dosages and chemotherapy induced toxicity (Butturini et al. 2007). Although, it remains unclear if obesity is associated with poorer outcomes, it is vital to reduce its prevalence to minimise long-term obesity related disorders, which are exacerbated by the side-effects from the treatment (Oeffinger et al. 2006a).

2.1.4.5 Limitations of study

Several limitations were identified in this review. During the search process, potential language bias might have occurred as the authors restricted the review to the English language. Following the risk of bias assessment using the CASP tool, it became clear that the quality of studies varied considerably, which goes some way to explaining the great differences in reported prevalence of malnutrition in this review.

2.1.5 Conclusion

This systematic review represents the first effort to date to review the epidemiological evidence on prevalence of malnutrition in children diagnosed with cancer worldwide, and to explore whether or not malnutrition is associated with clinical outcome. Unfortunately, these aims have been largely frustrated by both the absence of high-quality research and the heterogeneous nature of the existing outcome data. The single most important conclusion from this systematic review is the need for future high-quality population based longitudinal cohort studies, which consider the recommendations highlighted here. Nonetheless, the present evidence raises the possibility that children diagnosed with and treated for cancer are likely to be undernourished and or overnourished at different stages of the disease.

2.1 SYTEMATIC REVIEW: PREVALENCE AND POSSIBLE CAUSES OF VITAMIN D DEFICIENCY AND INSUFFICIENCY IN PAEDIATRIC CANCER PATIENTS

2.2.1 Introduction

Survival rates of pediatric cancer patients have improved considerably in the last 40 years as a result of treatment advances (Herbst et al. 2006). Consequently, attention is shifting to the reduction of treatment-related sequelae during and after the completion of therapy. Vitamin D inadequacy is now an international recognised health problem (Ahmed et al. 2011); however, there is still debate about the definitions of deficiency, insufficiency and sufficiency (Holick 2004, RCPCH 2013, Holick et al. 2011, Holick 2009) and, guidelines on supplementation remain inconsistent (Pramyothin and Holick 2012). Worldwide, one in seven people (14%) are estimated to have either insufficient or deficient vitamin D status (Holick 2007), whilst in healthy children, the prevalence ranges from 14% to 49% (Choudhary et al. 2013). Childhood cancer patients may be at even higher risk of vitamin D deficiency than the healthy population (Duncan et al. 2011), as prevalence has been reported as high as 80% in children diagnosed with acute lymphoblastic leukaemia (ALL) (Atkinson 2008). Additionally, survivors of childhood cancer have an increased risk of developing the metabolic syndrome, cardiac complications and have a reduced peak bone mass due to related treatment side-effects (Wallace et al. 2013), which might be exacerbated by vitamin D deficiency during and after the completion of therapy (Holick 2004).

Vitamin D is primarily obtained from sunlight (endogenously synthesised from a zoosterol 7-dehydrocholesterol) and it can also be absorbed directly from the diet, however very few foods contain naturally occurring vitamin D (Holick 2006). Once absorbed, vitamin D_2 or vitamin D_3 are converted to 25-hydroxyvitamin D_2 ($25(OH)D_2$) or 25-hydroxyvitamin D_3 ($25(OH)D_3$) metabolites in the liver, which may then be activated in the kidneys to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (figure 3.3) (Holick 2006). The functions of vitamin D in the body are often divided into musculoskeletal and non-musculoskeletal. Until now, the principal functions of vitamin D were held to lie in the musculoskeletal system as vitamin D is essential to

allow optimal calcium homeostasis, adequate growth and skeletal development in children. Consequently, prolonged periods of vitamin D deficiency in children increase the risk of bone fractures, rickets and of slow growth (Holick et al. 2011), with the subsequence increase risk of osteoporosis in later life (Oeffinger et al. 2006b). More evidence is emerging supporting a wider role for vitamin D beyond the musculoskeletal system (Holick 2010). A potential role in lowering both blood pressure and the risk of developing Diabetes Mellitus has been reported (Holick 2010, Huh and Gordon 2008). Work is also ongoing exploring its anti-inflammatory and immunomodulatory effects, which allied with potential cell growth regulatory functions (Holick 2010, Huh & Gordon 2008), raises the possibility of a role in preventing cancer progression (Holick & Chen 2008).

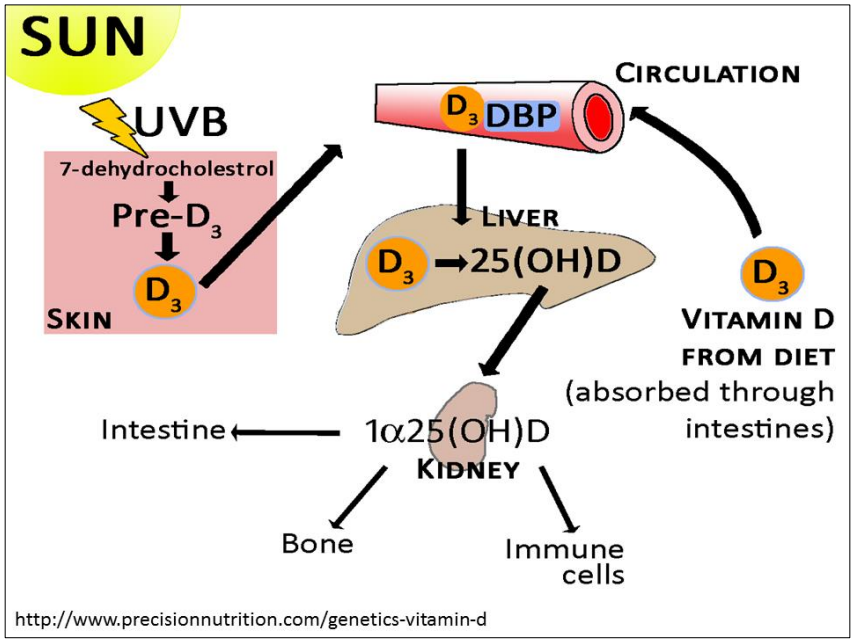

Figure 2.3 Absorption, metabolism and functions of vitamin D

(Obtained with permission from http://www.presionnnutrition.com/genetics-vitamin-d)

Children diagnosed with cancer may be at increased risk of vitamin D deficiency due to the disease itself, but also the multiple treatment induced side-effects (Duncan et al. 2011). In normal circumstances, vitamin D binding protein (DBP), which is synthesised in the liver, binds to vitamin D (25(OH)D and 1,25(OH)D) and

transports these to target tissues (White & Cooke 2000, Speeckaert et al. 2006). Recent evidence postulates that plasma concentrations of albumin and DBP are correlated (Speeckaert et al. 2006). The concentration of plasma DBP, like albumin, might be reduced during the disease process, reducing in turn the body's ability to transport vitamin D to target tissues (Speeckaert et al. 2006, Speeckaert et al. 2006). Some cytotoxic drugs, radiotherapy and antifungals cause phototoxicity. Thus, children who receive these treatments are advised to avoid sunlight (Duncan et al. 2011). Some chemotherapy agents can cause hepatotoxicity and acute and or chronic kidney injury, thereby interfering with the activation of vitamin D from its inactive ($25(OH)D_2$ and D_3) to its active form ($1,25(OH)_2D$) (Atkinson 2008). Glucocorticoids stimulate vitamin D catabolism and can increase the risk of vitamin D deficiency (Zhou et al. 2006). Additionally, the long term use of glucocorticoids increase the risk of bone fractures, which in turn may be exacerbated with vitamin D deficiency (Holick 2006). Mucositis is a common complication of cancer therapy, which results in reduced absorption of essential vitamins and minerals, including vitamin D (Atkinson 2008). Finally, impaired growth velocity may occur during treatment of childhood cancer (Wallace et al. 2013), which can also be exacerbated by vitamin D deficiency, as this is essential to allow adequate growth and skeletal development in children (Holick 2006).

Given that most children and adolescents now survive into adulthood (Wallace et al. 2013), the emerging evidence of the importance of vitamin D on health and the absence of a published systematic review looking at this subject, it is now essential to study the epidemiological picture of vitamin D deficiency in paediatric cancer patients. Thus the aims were (i) to establish the prevalence of vitamin D deficiency and insufficiency in children and adolescents diagnosed and treated for cancer at different stages of the disease (diagnosis, during treatment and at the end of therapy) and (ii) to explore possible causes of vitamin D inadequacy in this population.

2.2.2 Methods

A protocol was designed a priori (http://www.crd.york.ac.uk/PROSPERO/ registration number: CRD42013004371). The process and reporting of this systematic review was performed according to the Cochrane Collaboration and following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al. 2009).

2.2.2.1 Outcome data

The primary outcome was

(i) Plasma/serum 25 hydroxyvitamin D (25(OH)D) defined as nmol/L or ng/ml.

The secondary outcomes were:

(i) Plasma/serum 1,25 dehydroxycholecalciferol (1,25(OH)D) defined as nmol/L or ng/ml.

(ii) Studies looking at plasma parathyroid hormone as well as plasma 25(OH)D and 1,25(OH)D levels.

(iii) Studies looking at possible causes of vitamin D inadequacy (deficiency and insufficiency).

2.2.2.2 Eligibility criteria and search strategy

The eligibility criteria included:

iii. Studies investigating children and young people, aged <18 years and diagnosed with cancer according to the International Classification of Childhood Cancer, third edition (ICCC-3)

iv. To minimise the risk of bias there was no restriction on follow up period

Electronic searches with no language restrictions were performed (no restriction-August 2013) using the Cochrane Library, MEDLINE (via EBSCOhost), CINAHL (via EBSCOhost), PUBMED and Google search to identify systematic reviews, Randomised Controlled Trials (RCT), observational studies, case control studies and letters to the editor. The reference list of all relevant articles, narrative reviews and private collections was also examined. The initial search strategy identified the

following keywords and subject heading searches (MeSH); “pediatrics”, “cancer”, “cancer treatment”, “vitamin D”.

2.2.2.3 Study selection, quality assessment and data extraction

Titles and abstracts from the combined searches were reviewed by two researchers independently (RRI, ER). In those cases of disagreement, an independent advisor (DW) made the final decision. Evidence was critically appraised independently by two researchers (RRI, IP) employing a standard methodological tool; the Critical Appraisal Skills Programme (CASP). In case of disagreement a third independent reviewer (DW) made the final assessment and a decision was made. The CASP tool has been recommended for epidemiological studies, public health and evidence based practice (Sanderson et al. 2007). The CASP comprises an assessment of: (i) Contextual information including the study objectives, study design and the patient’s characteristics; (ii) Potential selection bias including inclusion and exclusion criteria, clear patient selection and an assessment of validity, reliability and accuracy of techniques used; (iii) Outcome measures including reference values; (iv) statistical analysis employed and (v) a reporting of results and control for confounding factors. Each section was classified as “yes” if the authors covered the questions, “can’t tell” if it was not reported or “no” if it was not covered or considered at all. A quality rating of “strong”, “moderate” or “weak” was applied to each study; where “strong” was given when the study met all areas of the CASP criteria, “moderate” when there was one weak area and “weak” when ≥ two weak areas were identified.

For those studies that did not report the prevalence of vitamin D deficiency and insufficiency, we contacted the corresponding author first on two occasions, giving two weeks in between emails to allow for some time to reply. If that failed, we then repeated the process by contacting the last or most senior author and then the second author. If no response was received after these three attempts, the article was included, however the data did not count towards the final prevalence results as this would have been unavailable. Finally, the performance of a meta-analysis of selected studies investigating the association between vitamin D inadequacy and vitamin D related treatment complications was planned a priori.

Studies were classified according to four diagnostic criteria: haematological malignancies, solid tumours, brain and benign tumours and all malignancies (ICCC-3) when studies considered all diagnosis together. Finally, we calculated the median and the range percentage of the prevalence of vitamin D inadequacy using the reference values recommended in the Endocrine Society Clinical Practice guideline (Holick et al. 2011), which defines 25(OH)D levels as sufficient/optimal (>75nmol/L or 30ng/ml), insufficient (75-50nmol/L or 30-20ng/ml) and deficient (<50nmol/L or <20ng/ml).

2.2.2.4 Meta-analysis

A meta-analysis investigating whether age was associated with vitamin D status was performed. Data on correlations and odds ratio of age with vitamin D deficiency and insufficiency were extracted from each relevant study and converted to a common effect size (ES), Fisher's Z and the associated standard error. These were combined in a meta-analysis using the Meta Procedure, with Stata version 9.2 to synthesise an overall ES and 95% Confidence Interval, and the associated Forrest plot. Statistical heterogeneity was assessed using the Q statistic, with $p<0.05$. The results of both fixed and random effects models were generated, with the resulting models being similar, hence only the fixed effects model is reported here. Variation in ES attributable to heterogeneity was assessed with the I-squared statistic, which is defined as the percentage of the study heterogeneity attributable to variability in the true treatment effect, rather than sampling variation.

2.2.3 Results

2.2.3.1 Study selection and study characteristics

19 studies met the eligibility criteria (figure 2.3). Of these, eight were prospective cohort studies (Arikoski et al. 1999a, Atkinson et al. 1998, Bilariki et al. 2010, El-Ziny et al. 2005, El-Ziny et al. 2007, Halton et al. 1996, Henderson et al. 1998, Rosen et al. 2013), six cross-sectional studies (Arikoski et al. 1999b, Atkinson et al. 1989, El-Hajj Fuleihan et al. 2012, Halton et al. 1995, Halton et al. 1995b, Modan-Moses et al. 2012, de Schepper et al. 1994) and three case-control studies (Frisk et al.

2012, Gunes et al. 2010, Sinha et al. 2011). There was also a retrospective cohort study (Choudhary et al. 2013) and a non-randomised control trial (Wiernikowski et al. 2005). Vitamin D levels in paediatric cancer patients were investigated either as a primary or secondary outcome. Ten studies investigated vitamin D levels in children diagnosed with haematological malignancies and acute lymphoblastic leukaemia was considered in all studies. Six studies considered children diagnosed with childhood cancers included in the ICCC-3, five studies considered solid tumours and two considered children diagnosed with either brain tumours or benign brain tumours. Finally, four studies investigated vitamin D status in children diagnosed with more than one cancer category.

A total of 1235 participants were included in all 19 studies with individual sample sizes ranging from 10 to 426. Four studies used the same population (Atkinson et al. 1998, Halton et al. 1996, Atkinson et al. 1989, Halton et al. 1995a). Thus, only subjects and data from one study (n=40) was accounted for within the total number of participants. The 19 studies were all written in English and eight were performed in North America, six in Europe and five in the Middle East. The methods used to analyze vitamin D status were reported in all studies, however only five studies (Frisk et al. 2012, Arikoski et al. 1999a, Arikoski et al. 1999b, El-Hajj Fuleihan et al. 2012, Sinha et al. 2011) reported intra assays' coefficient of variation, which ranged from 3.6 to 14% (table 3.11).

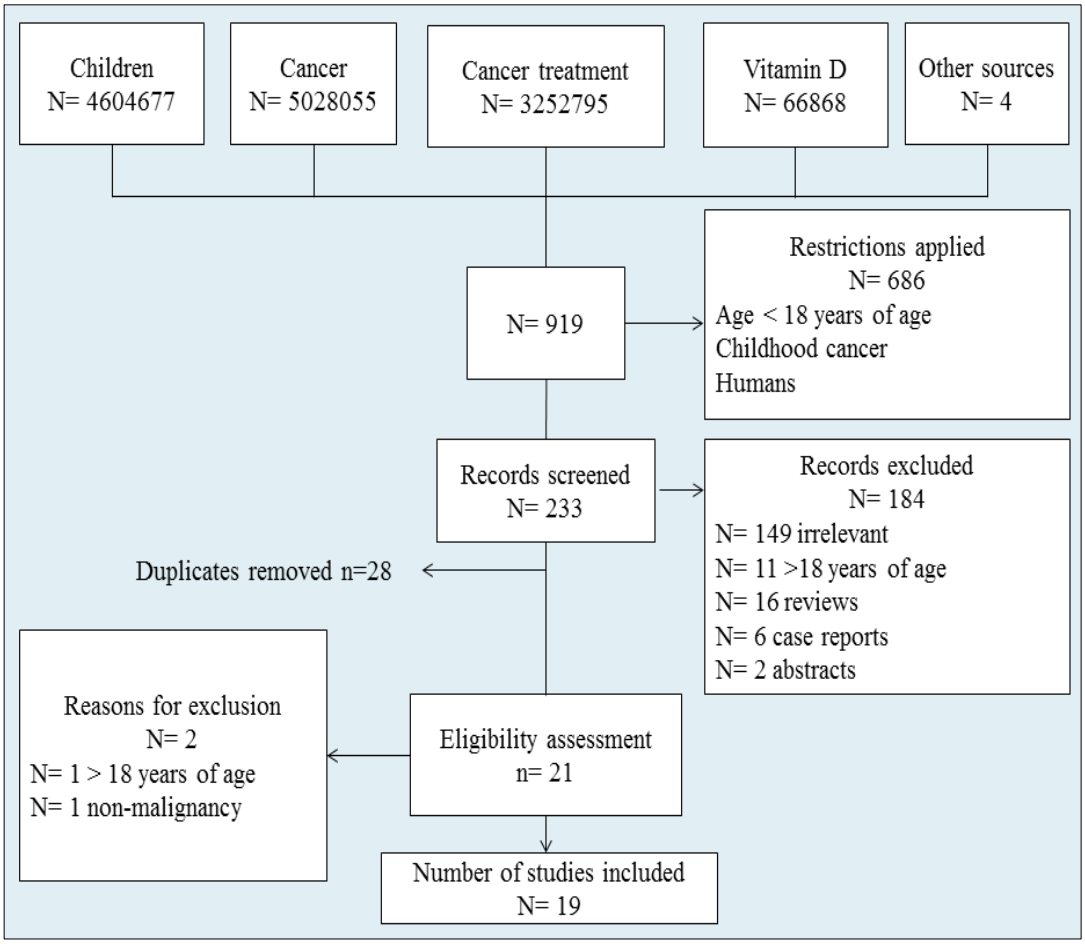

Figure 2.4. Flow chart of the studies screened, selected, assessed for eligibility criteria and included in the systematic review.

2.2.3.2 Prevalence of vitamin D deficiency and insufficiency

Plasma 25(OH)D and 1,25(OH)D are presented separately as these two metabolites provide different clinical information: 25(OH)D is the best marker of vitamin D status in humans (Zerwekh 2008), whilst 1,25(OH)D is tightly regulated by calcium homeostasis and its concentration changes rapidly (Zerwekh 2008).

The prevalence of plasma 25(OH)D status was reported in 13/19 studies (table 2.11; table 2.12). Overall, the median prevalence of vitamin D inadequacy was 37% (range 0-83%). Of this, prevalence of vitamin D deficiency was 14% (range 0-61.5%) and insufficiency 23% (range 0-83%). In order to assess plasma 25(OH)D status, the results were separated into three different groups; those studies reporting results at

diagnosis, during treatment and after the completion of therapy (figure 2.5). No study reported prevalence at three stages of the disease; diagnosis, treatment and after the completion of therapy.

Eight out of 19 studies assessed 1,25(OH)D (table 2.11) and the prevalence of 25(OH)D was stratified according to diagnostic category (table 2.12 and figure 2.6) and geographical location (by continent) (table 2.13; figure 2.6).

PTH measured alongside vitamin D is considered a more sensitive physiological measure of vitamin D status in the healthy population (Holick et al. 2009); therefore this was also assessed. Twelve out of 19 studies (Atkinson 2008, Arikoski et al. 1999a, Bilariki et al. 2010, El-Ziny et al. 2005, El-Ziny et al. 2007, Halton et al. 1996, Arikoski et al. 1999b, El-Hajj Fuleihan et al. 2012, Sinha et al. 2011, Halton et al. 1995a) investigated plasma PTH levels as well as 25(OH)D or 1,25(OH)D (table 3.1). However, only 5 studies reported plasma PTH in relation to either 25(OH)D or 1,25(OH)D with very heterogeneous findings: (i) Bilariki et al. (2010) reported hyperparathyroidism in half of patients (n=15) who were 25(OH)D deficient (n=32); (ii) Atkinson et al. (1989) reported normal plasma PTH and 25(OH)D in all ALL patients; (iii) Halton et al. (1996) stated that 9 children with low 1,25(OH)D had also developed hyperparathyroidism; (iv) Schepper et al. (1994) found normal plasma 1,25(OH)D levels, however 15% (2/13) of these had hyperparathyroidism and 8% (1/13) hypoparathyroidism; (v) Finally, Sinha et al. (2011) did not establish any significant associations between plasma 25(OH)D and PTH levels in pediatric cancer patients ($r=0.07$; $p=0.65$); but this association was significant in the control group ($r=-0.42$; $p<0.01$).

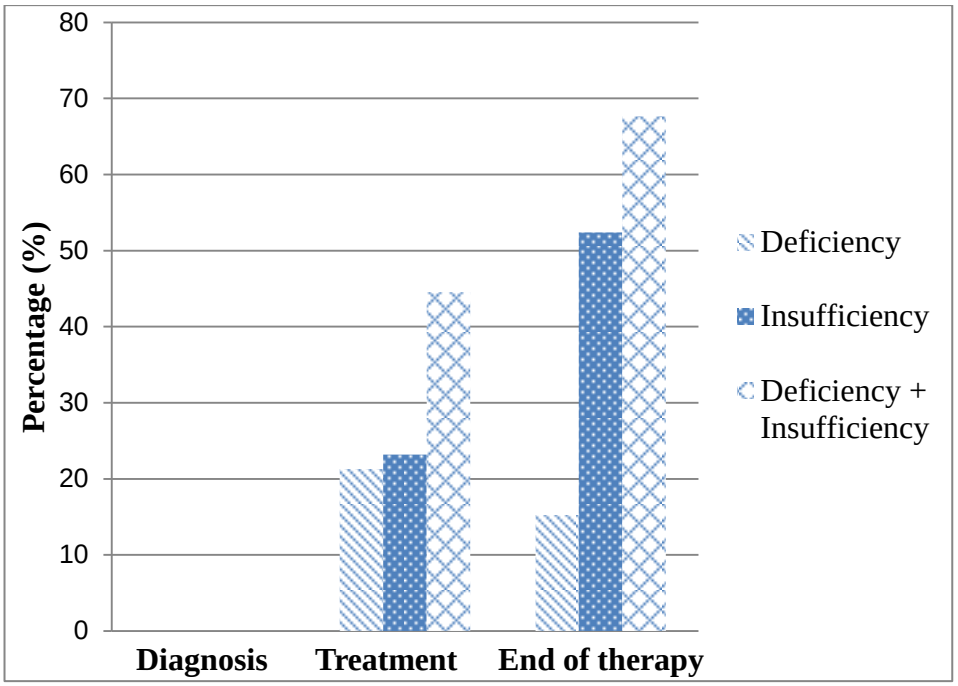

Figure 2.5 Prevalence of vitamin D (25(OH)D) deficiency and insufficiency according to stage of disease (diagnosis, treatment and end of therapy)

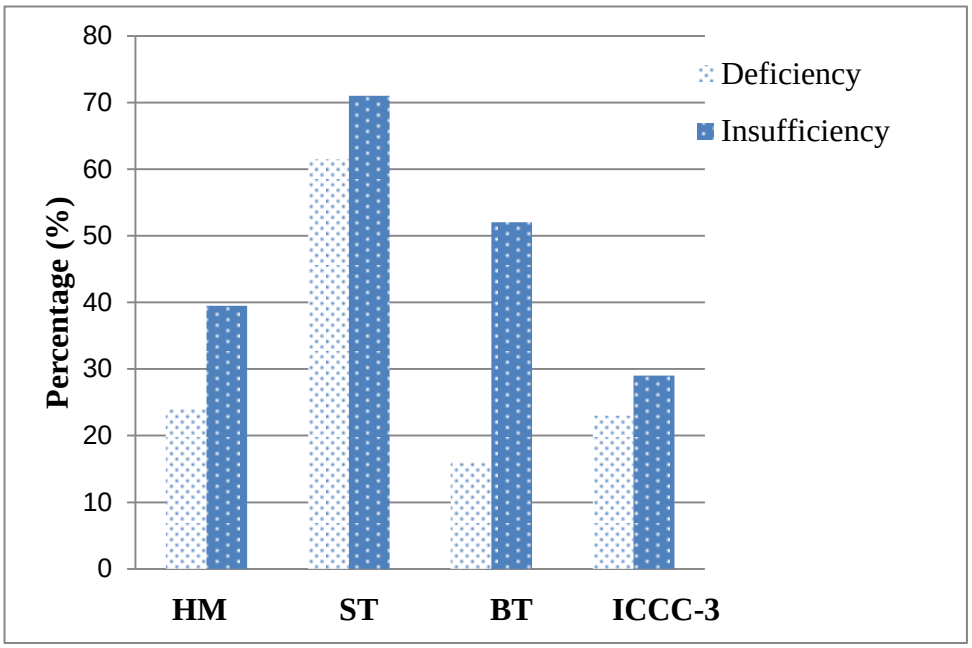

Figure 2.6 Prevalence of vitamin D (25(OH)D) deficiency and insufficiency categorised by diagnostic category

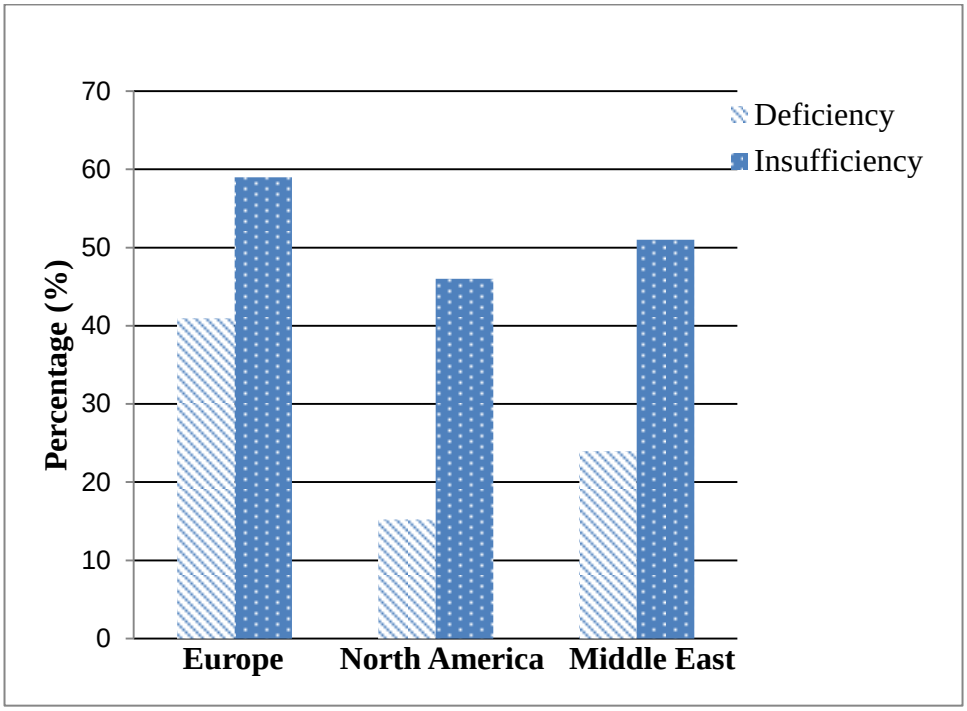

Figure 2.7 Prevalence of vitamin D deficiency and insufficiency categorised by continent

Table 2-11 Studies reporting prevalence of vitamin D status in paediatric cancer patients

Author	Quality	Patients Dx	N, age at diagnosis (years)	Method/time of measurements	Method		Results		
					Design	Parameters/Outcome	At diagnosis	During treatment	After treatment
(Arikoski et al. 1999a) Finland	Moderate	Hematological malignancies and solid tumors	N= 28 Median (range) 8 (2.9-16)y HM (n=10) ST (n=18)	M: Parviainen et al 1981 Assay's coefficient of variation: 8-14% PTH two-site immunoradiometric assay Assay's coefficient of variation: NR T: at diagnosis, 3 months, 6 months and 1 year	PC	25 (OH)D nmol/L 1,25-$(OH)_2$D pmol/L Values compared against Finish children PTH reference data: NR Ethnicity: Caucasian (n=28)	25 (OH)D nmol/L Mean (±SD) 30 (±11)* 1,25-$(OH)_2$D pmol/L Mean (±SD) 76 (±56) Prevalence NR PTH levels were sig decreased compared to normal values Mean (±SD) and prevalence: NR	1 year follow up 25 (OH)D nmol/L Mean (±SD) 25 (±12)* 1,25-$(OH)_2$D pmol/L Mean (±SD) 73 (±44)* Prevalence NR PTH levels were sig increased at 6 and 12 months compared to diagnosis Mean (±SD) and prevalence: NR	
(Arikoski et al. 1999b) Finland	Moderate	Childhood malignancies	N= 48 Median (range) 10.2 (3.6-17.8)y HM (n=25) ST (n=22) BT (n=1)	M: Parviainen et al 1981 Assay's coefficient of variation: 8-14% PTH standard methods T: one month after treatment	CS	25 (OH)D nmol/L 1,25-$(OH)_2$D pmol/L Values compared against Finish children PTH reference data: NR Ethnicity: Caucasian			25 (OH)D nmol/L Mean (±SD) 34 (±18)** 1,25-$(OH)_2$D pmol/L Mean (±SD) 67 (±33)** Prevalence NR PTH Mean (±SD) 42.4 (±24.0) ng/L Prevalence:

									1/46 (2%) low 4/46 (9%) high
(Atkinson et al. 1989) Canada	Weak	ALL	N=16 Mean (±SD) 6.2(±1.5)y	M: High-pressure liquid chromatography D_3 and radiochemical assays Frayer LJ et al 1983 Assay's coefficient of variation: NR PTH two-site immunoradiometric assay (N-tact PTH IRMA KitINCSTAR Corp., Stillwater, MN, USA) T: after first remission and 6 months after completion of treatment I: magnesium supplementation	Time series-CS	Reference data: 25(OH)D (5.0-50) ng/ml 24,25-(OH)2D (0.5-5.0) pg/ml 1,25-(OH)2D (20-65) pg/ml PTH <0.88 units/L Values compared against Frayer et al. 1983 Ethnicity: NR		0% deficiency on 25(OH)D ng/ml 3/16 (19%) elevated 24,25(OH)D 12/13 (92%) non-detectable plasma $1,25(OH)_2$ D PTH normal range	0% deficiency on 25(OH)D ng/ml Elevated 24,25 (OH) D NR 7/14 (50%) non-detectable plasma $1,25(OH)_2D$ PTH normal range
(Atkinson et al. 1998) Canada	Moderate	ALL	N=40 Median (range) age 3.9 (0.3-17)y	M: High-pressure liquid chromatography D_3 and radiochemical assays (Fraher et al 1983) Assay's coefficient of variation: NR PTH two-site immunoradiometric assay (N-tact PTH IRMA KitINCSTAR Corp., Stillwater, MN, USA) T: 1st diagnosis/pre-therapy (0-5 days) 2nd post-induction steroid, 6 months 3rd post-initiation of therapy; 12 months 4th 24 months	PC	Plasma $1,25-(OH)_2D$ pmol/L Normal range (48-156) pmol/L PTH reference data (0.3-3.5) pmol/L Ethnicity: NR	Plasma 1,25-(OH)2D Mean (±SD) 33.4±26.4 pmol/L Prevalence of 25(OH)D deficiency: NR PTH Median (range) 1.4(0.8-1.7)	6 months 1,25-(OH)2D Mean (±SD) 29.3±(26.4) 64% had a reduction from baseline PTH: NR 12 months Mean(±SD) 36.2±(SD) Prevalence of vitamin D deficiency: NR PTH: NR PTH at 31 days Median (range) 5.1 (1.5-11.4)	Plasma 1,25-(OH)2D 24 months Mean(±SD) 35.0±21.6) Prevalence of vitamin D deficiency: NR PTH: NR

				I: magnesium supplementation					
(Bilariki et al. 2010) France	Moderate	Solid and brain tumors	N= 52 Median (range) age at diagnosis 9.0 (0.7-18.5)y ST (n=48) BT (n=4)	M: 25(OH)D and 1,25(OH)D radioimmunoassays (Diasorin, Sallugia, Italy) Assay's coefficient of variation: NR PTH Chemiluminescence (Elecsys, Roche Diagnostics, Meylan, France) T: 1st after completion of therapy (within 2 years after treatment) 2nd 1 year following first measurement	PC	Plasma 1,25(OH)D ng/ml Plasma 25(OH)D ng/ml Reference: Cormier and Souberbielle 2006 Deficiency: <20ng/ml Insufficiency: 20-30ng/ml PTH reference: 10-46pg/ml Ethnicity: NR	NA	NA	Plasma 25 (OH)D ng/ml 1st measurement: (48/52 patients): 25(OH)D Mean (±SD) 19.7±8.5ng/ml Below suboptimal: (<30ng/ml): 83% Deficiency: (<20ng/ml): 61.5% Severe deficiency: (<10ng/ml): 11.5% 2nd measurement (20/52) Mean (±SD) 20.5(±7.1)ng/ml Deficiency: (<20ng/ml): 55% Plasma 1,25(OH)D NR PTH Mean (±SD) 45.5(±21) pg/ml Of those who were vitamin D deficient half developed hyperparathyroidism
(Choudhary et al. 2013) USA	Strong	Cancer and SCT patients	N= 426 cancer N=58 SCT Median (range) 3.9 (0-18)y HM (n=85) ST (n=212)	M: 25-OH D Chemiluminescent (Diasorin) assay Assay's coefficient of variation NR T: Median (range) interval between diagnosis and 25-OH D test 12.3 (2.05-	RC	25-OH D insufficiency <20ng/ml Guidelines Institute of Medicine (IOM) PTH: NA Ethnicity:	NA	NA	Insufficient: 29%

			BT (n=114) Other (n=15)	36.4)y		White (n=381) Black (n=59) Hispanic (n=3) Asian (n=41)			
(El-Hajj Fuleihan et al. 2012) Lebanon	Strong	Hematological malignancies	Patients n= 29 Mean(±SD) 9 (±2.9)y Controls n=32 Mean (±SD) 8.8(±2.6)y	M: 25-OH D Competitive protein binding assay RIA (Immuno-Diagnostic Systems, Boldon,UK) Assay's coefficient of variation: 5-8.2% PTH ELSA-PTH, IRMA (immunoradiometric assay) Assay's coefficient of variation: 1-5.5% T: Mean (range) 14 (12-17) months from diagnosis	CS	25-OH D <10ng/ml 25-OH D <20ng/ml 25-OH D >25ng/ml Reference data NR PTH reference data NR Ethnicity: NR	NA	25(OH) D <10ng/ml Patients: 24.1% Controls: 12.5% 25(OH)D <20ng/ml Patients: 79.3% Controls: 62.5% 25(OH) D >25ng/ml Patients: 21% Controls: 16% Controls v cases p=0.08 PTH Mean (±SD) Patients 52(±27) pg/ml Controls 21(±12) pg/ml	NA
(El-Ziny et al. 2005) Egypt	Weak	AML and ALL	N=43 Mean (±SD) 7.0(±3.9)y Vitamin D sample: Cases n=20 Healthy controls n=12	M: 25(OH)D_3 RIA-Biosource Europe SA, Nivelles, Belgium) Assay's coefficient of variation: NR PTH PTH-EASIA (ELIZA-Biosource Europe S.A, Nivelles, Belgium) Assay's coefficient of	PC	25(OH)D_3 ng/ml cases compared to healthy controls Reference data NR PTH reference data: NR Ethnicity: NR	25(OH)D_3 ng/ml Cases: At presentation: median (IQR): 11 (5.3-29) Controls: Median (IQR) 66 (61-69)	25(OH) D ng/ml 3 months: 14.2 (5.5-26.8) 12 months: 17.5 (10.3-38.5)	NA

				variation: NR T: At presentation, after induction CT and 12 months after therapy				$25(OH)D_3$ (ng/ml) was significantly lower in ALL in comparison to controls ($p<0.001$) Prevalence NR PTH normal levels in all cases and no sig difference between cases and controls	
(El-Ziny et al. 2007) Egypt	Weak	Hodgkin and non-Hodgkin Lymphoma	N=20 Mean (±SD) 8.9±(4.2)y Vitamin D sample: Cases n=20 Healthy controls n=12	M: $25(OH)D_3$ RIA-Biosource Europe SA, Nivelles, Belgium) Assay's coefficient of variation: NR PTH PTH-EASIA (ELIZA-Biosource Europe S.A, Nivelles, Belgium) Assay's coefficient of variation: NR T: At presentation, after induction CT and 12 months after therapy	PC	$25(OH)D_3$ ng/ml cases compared to healthy controls Reference data NR PTH reference data: NR Ethnicity: NR	$25(OH)D_3$ ng/ml Cases: At presentation: median (IQR): 8.5 (6.7-21.0) Controls: Median (IQR) 66 (61-69)	$25(OH)D_3$ ng/ml 3 months: 29.0 (16.0-49.0) 12 months: 12.0 (10.0-29.0) $25(OH)D_3$ (ng/ml) was significantly lower in HL & NHL in compare to controls ($p=0.001$) Prevalence NR PTH normal levels in children with lymphoma	NA

								and no sig difference between cases and controls	
(Frisk et al. 2012) Sweden	Moderate	ALL and lymphoblastic lymphoma and SCT	N=15 Cases: Age at diagnosis Median (range) 9.8 (5.6-18.3)y Age at study Median (range) 27.5(17.3-37.1)y Controls: Age at study Median (range) 27.3(18.9-38.6)y	M: Chemoluminiscence (DiaSorin, Liaison, Saluggia, Italy) Assay's coefficient of variation: 3.6-7.1% T: 10 years after SCT	CC	25(OH)D < 37nmol/L hypovitaminosis D Reference data Kananen et al. 2002, Taskinen et al. 2006 PTH: NA Ethnicity: NR	NA	NA	Cases: 59% Controls: 22% 25(OH)D was significantly lower in the cases group than in the control group p<0.05
(Gunes et al. 2010) Turkey	Moderate	ALL	N=70 At diagnosis Mean (±SD) 5.7(±3.4)y At enrolment Mean (±SD) 10.6(±3.8) y Age and sex matched control group	M: Radioimmunoassay 25OH-VIT.D-RIA-CT KIP 1961) Assay's coefficients of variation: NR T: at the end of ALL therapy	CC	25(OH)D ng/ml cases compared to healthy controls Reference data NR PTH: NA Ethnicity: NR	NA	NA	25(OH)D ng/ml was significantly lower in the case group than in the healthy controls (p=0.03) Prevalence NR
(Halton et al. 1995a) Canada	Moderate	ALL	N=40 Median (range) 3.9 (0.3-17)y	M: High-pressure liquid chromatography D_3 and radiochemical assays (Fraher et al 1983) Assay coefficient of variation: NR PTH: PTH two-site	CS	25(OH)D_3 reference data (Fraher et al. 1983) 1,25$(OH)_2D_3$ reference data (Fraher et al. 1983): Low (<48 pmol/L or <20pg/ml)	25(OH)D_3 normal status 1,25$(OH)_2D_3$ Low 30 (75%) Low normal 8	NA	NA

				immunoradiometric assay (N-tact PTH IRMA KitINCSTAR Corp., Stillwater, MN, USA) T: at diagnosis		Low- normal (48-62.4 pmol/L or 20-26pg/ml) Normal (91.2-139pmol/L or 38-58pg/ml) PTH reference data 0.5-5.0 pmol/L Ethnicity: NR	(20%) Normal 2(5%) PTH (2.0±1.9) pmol/L Prevalence: Low 14% High levels NR		
(Halton et al. 1996) Canada	Moderate	ALL	N=40 Median (range) 3.9 (0.3-17)y	M: High-pressure liquid chromatography D3 and radiochemical assays (Fraher et al 1983) Assay's coefficient of variation: NR PTH two-site immunoradiometric assay (N-tact PTH IRMA KitINCSTAR Corp., Stillwater, MN, USA) T: At diagnosis, 6, 12, 18 and 24 months	PC	25(OH) D_3 reference data (Fraher et al. 1983) 1,25(OH)2D3 reference data (Fraher et al 1983): Low (<48 pmol/L or <20pg/ml) Low normal (48-62.4 pmol/L or 20-26pg/ml) Normal (91.2-139pmol/L or 38-58pg/ml) PTH Normal range (0.5-5.0 pmol/L) Ethnicity: NR	25(OH)D_3 normal status $1,25(OH)_2D_3$ >70% below normal range PTH Mean (±SD) 2.0(±1.9)	25(OH)D_3 normal status $1,25(OH)_2D_3$ 70% below normal range PTH did not vary sig throughout treatment At 24 months 9 children with low $1,25(OH)_2D_3$ had elevated plasma PTH	NA
(Henderson et al. 1998) USA	Weak	Malignancy treated with CT	N=37 Mean (±SD) 7.3(±4.5)y range (1.4-16.9) y ALL (n=24) ST (n=13)	M: Assay kit (Incstar, Stillwater, MN) Assay's coefficient of variation: NR T: followed up between 12 and 41 months How often NR	PC	25(OH)D <15ng/ml low 1,25(OH)D <20pg/ml low Reference data (Chesney 1981, Taylor et al. 1984) PTH: NA Ethnicity: NR	25(OH)D deficiency 1/37 (3%) 1,25(OH)D 0/37(0%)	25(OH)D deficiency 1/37(3%) 1,25(OH)D 0/37(0%)	NA
(Modan-Moses et al. 2012) Israel	Moderate	Malignancy	N=142 Median (range) 12.03 (1.1-5.8)y	M: DiaSorin LLAISON competitive two-step chemiluminescent immunoassay (DiaSorin, Stillwater, MN)	CS	25(OH)D status (reference data Misra et al. 2008) Optimal: >32ng/ml	NA	Optimal 10 (7%) Sufficient 74 (52.1%)	NA

			ALL (n=57) ST (n=53) BT (n=32)	Assay's coefficient of variation: NR T: one measurement at clinic during or soon after the completion of treatment		Sufficiency: >20 ng/ml Insufficiency: 15-20 ng/ml Deficiency: <15 ng/ml PTH: NA Ethnicity: NR		Insufficiency 33(23.2%) Deficiency 35(24.6%)	
(Rosen et al. 2013) USA	Moderate	Benign tumor Malignancy Treated with combination of surgery, CT and/or RT	N= 201 Mean (±SD) at diagnosis 5.6(±0.3)y Mean (±SD) at initial visit 5.4 (±0.2)y	M: Liquid chromatography/tandem mass spectrometry (LC-MS/MS) by Sonora Quest Laboratories (Quest Nichols, san Juan Capistrano, CA) Assay's coefficient of variation: NR T: two assessments 1st at study entry 2nd two years after completion of therapy	PC	25(OH)D status (reference The Endocrine Society (Holick et al. 2011) Sufficient: >30 ng/ml Insufficiency: 20-29.9 ng/ml Deficiency: <20 ng/ml PTH: NA Ethnicity: Caucasian (n=130) Hispanic (n=51) African-American (n=5) Asian-American (n=3) Native-American (n=9) Other (n=3)	NA	NA	1st Evaluation (n=201): Sufficient 46.3% Insufficiency 39.3% Deficiency 14.4% 2nd Evaluation (n=124): Sufficient 31.5% Insufficiency 52.4% Deficiency 16.1% Of the 124 patients that had 25(OH)D concentration was reduced by 11.4%**
(de Schepper et al. 1994) Belgium	Weak	Malignancy	N=13 Median (range) 9.5(2.9-17.4)y ALL (n=2) ST (n=11)	M radioimmunoassay Nicholas Institute, San Juan Capistrano, CA, USA) Assay's coefficient of variation: NR PTH assay method NR T: once, minimum 3 months after CT	CS	Parameter 1,25(OH)$_2$ vitamin D$_3$ Normal range 50-150 nmol/L Reference data NR PTH normal range 10-35ng/L Ethnicity: NR	NA	NA	1,25(OH)D 0% deficiency PTH 1/13 (8%) low 2/13 (15%) high
(Sinha et al. 2011) UK	Moderate	Pediatric cancer	Cases N=61 Median (range) 11.1 (1.5-24.4)y HM (n=19) ST (n=21) Others (n=8) BT (n=13)	M DiaSorin 25-hydroxyvitamin D radio-immunoassay (RIA) (Cat #68100E Stillwater, Minnesota) Assay's coefficient of variation: 8.4-12.6% PTH Centaur Chemiluminometric	CC	25(OH)D Reference data (Weng et al. 2005, Vieth et al. 2007; Bischoff et al. 2006) Optimal: >75nmol/L Adequate: 50-75nmol/L Insufficiency: 25-50nmol/L Deficiency : <20 ng/ml	NA	Cases: Optimal: 9(14.7%) Adequate: 14 (22.9%) Insufficiency: 25 (40.9%) Deficiency: 13 (21.3%)	The median 25(OH)D concentration was sig lower in cases (44nmol/L) than in controls (52nmol/L)* Higher PTH levels were associated with

			Controls N=60 Median (range) 8.4(0.2-18.0)y	immunoassay Assay's coefficient of variation: 7.4-16.7% T: Once, median (range) time post diagnosis 3.0(0.4-9.0) y *Some patients were on treatment and some had finished*		PTH reference data NR Ethnicity (cases): Pigmented skin (n=3) Fair skin (n=58) Ethnicity (control): Pigmented skin (n=7) Fair skin (n=53)		PTH: NR The 3 pigmented skin children from the cohort were severely deficient compared to 1/7 in the control group Controls: Optimal: 8(13.3%) Adequate: 24 (40%) Insufficiency: 26 (43.3%) Deficiency: 2 (3.3%)	sig lower 25(OH)D in controls (r=-0.42; p<0.01) but not in cases (r=-0.07; p=0.65)
(Wiernikowski et al. 2005) Canada	Weak	ALL and NHL and on maintenance phase of CT	N= 10 Median (range) 7.2 (3.6-14.6)y	M: Radioimmunoassay (DiaSorin, Stillwater, MN, USA) Assay's coefficient of variation: NR T: week 0, 1, 4,7,10,13 and at the end of the study.	NRCT	**Intervention**: Alendronate orally once a week for 6 months Dose age dependant <8 years: 30 mg/week 8-10 years: 50 mg/week >10 years: 70 mg/week **Parameter:** Reference data 25(OH)D American adults (Bischoff-Ferrari 2004) Normal 9-37ng/ml Ethnicity: NR	0%	0%	NA

Dx: Diagnosis; M: method; T: time of measurement; C: cohort study; PC: prospective cohort study; RC: retrospective cohort study; CS: cross-sectional study; CC: case-control study; CS: cross-sectional; NRCT: non-randomised clinical trial; NR: not reported and/or not clear; NA: non-applicable (i.e. not part of study aims); M: method; T: time of measurement; Dx: Diagnosis; ALL: acute lymphoblastic leukaemia; HM: Haematological malignancies; NHL: non-Hodgkin's lymphoma; Treatment: CT: chemotherapy; R: radiotherapy; SCT: stem cell transplant; ng: nanograms; nmol: nanomols; pmol; picomols; pg: picograms; L: litre; mL: millilitres; SD: standard deviation; y: years; Countries: UK: United Kingdom; USA: United States of America; *p<0.05; **p<0.0001 compared to reference values; sig: significant.

Table 2-12 Prevalence of plasma 25(OH)D status in paediatric cancer patients

25(OH)D	Deficiency			Insufficiency		
	N of studies	Median %	Range %	N of studies	Median %	Range %
At diagnosis	3	0	0-3	4	0	0-25
During treatment	5	21	0-25	5	23	0-79
End of treatment	4	16	0-61.5	4	52	39-83
Overall	11	14	0-61.5	13	23	0-83

Table 2-13 Studies reporting the prevalence of plasma 25(OH)D deficiency and insufficiency stratified by location (continent)

25(OH)D	Deficiency			Insufficiency		
	N of studies	Median %	Range %	N of studies	Median %	Range %
Europe	2	41	21-61.5	3	59	41-83
Middle East	2	24	24-25	2	51	23-79
North America	8	15	0-16	4	46	39-52

Table 2-14 Prevalence of vitamin D (25(OH)D) deficiency and insufficiency according to diagnostic criteria

25(OH)D	Deficiency			Insufficiency		
	N of studies	Median %	Range %	N of studies	Median %	Range %
Haematological malignancies	3	24	0-75	5	39.5	0-79
Solid tumours	1	61.5	61.5	2	71	59-83
Brain and benign tumours	2	16	16-69.5	2	52	39-83
ICCC-3	5	25	21-29	2	32	23-41

2.2.3.3 Possible causes of vitamin D deficiency/insufficiency in paediatric cancer patients

Six out of 19 studies were found (table 2.15) exploring possible causes of vitamin D inadequacy in paediatric cancer patients. The outcome measured was 25(OH)D and the variables measured were: (i) demographic data: ethnicity, gender, age and pubertal status at diagnosis; (ii) clinical data: diagnosis, leukocyte count, length of treatment and nutritional status; (iii) different forms of cancer treatment including, the use of steroids, chemotherapy, radiotherapy and stem cell transplant (SCT) (iv) and environmental factors included, seasonal variation, sun exposure and the use of sun cream. A meta-analysis was performed with the variables age and vitamin D status only, as there was not commonality of independent variables; therefore, this evidence was described narratively.

The Forest plot depicting the included studies (vitamin D deficiency) is shown in Figure 2.7. For the vitamin D deficiency, the summary ES was -0.132 (95%CI -0.203, -0.060). All the studies showed a significant effect of younger age with higher vitamin D levels, with most ESs falling in the range -0.085 to -0.245. There is no evidence of heterogeneity (chi-squared =2.75 (d.f. = 2) P = 0.253), with the variation in ES attributable to heterogeneity (I-squared) = 27.3%. This level of heterogeneity indicates that there is little difference between the studies which cannot be explained by random variation.

Three studies considered whether ethnicity was associated with decreased 25(OH)D status. Of these, two (Choudhary et al. 2013, Sinha et al. 2011) reported associations between dark skin and decreased 25(OH)D status and one (Rosen et al. 2013) did not find such association. Seasonal variation was explored in three studies, two (Modan-Moses et al. 2012, Sinha et al. 2011) of which found that children who had their 25(OH)D measured in the Winter months had lower levels than those who were measured in the Summer months. Additionally, the use of sun cream was found to be associated with lower 25(OH)D status (Modan-Moses et al. 2012). Three studies investigated whether cancer diagnosis was associated with 25(OH)D and, of these, two (Rosen et al. 2013, Sinha et al. 2011) found that patients diagnosed with cancer

had lower 25(OH)D status than controls. The use of glucocorticoids was also investigated in two studies, which reported contradictory results. One (Arikoski et al. 1999b) found that the use of glucocorticoids was associated with lower 25(OH)D status and the other study (Choudhary et al. 2013) did not find such an association. Finally, none of the other variables were found to influence 25(OH)D status.

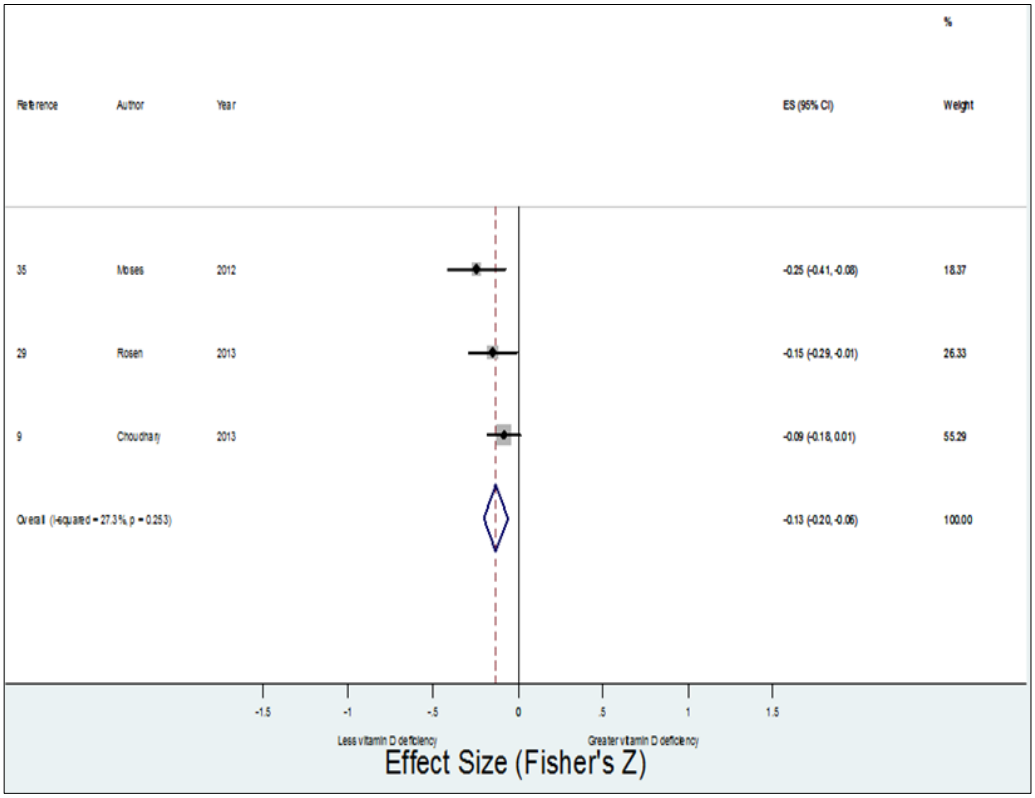

Figure 2.8 Associations between younger age and vitamin D inadequacy in paediatric cancer patients

The horizontal lines in the Forest plot represent the 95% confidence interval around the point estimate. The box around the point estimate is proportional to that of the study's weight in the analysis. The pooled estimate diamond is centred on the pooled estimate.

Table 2-15 Possible causes of vitamin D deficiency/insufficiency

Studies	Quality	Patients Dx	N, age at diagnosis (years)	Method/time of measurements	Method			Results
					Design	Variables	Outcome	Possible causes of vitamin D deficiency
(Arikoski et al. 1999b) Finland	Strong	Childhood malignancies	N= 48 Median (range) 10.2 (3.6-17.8)y	M: Parviainen et al. 1981 T: one month after treatment	CS	Use of steroids Bone turnover CT ALT	Low plasma vitamin D status: 25(OH)D nmol/L $1,25(OH)_2D$ pmol/L	Correlation: - steroids (r= 0.31; *p*= 0.04) No correlations: - Bone turnover - CT - ALT
(Choudhary et al. 2013) USA	Strong	Cancer and SCT patients	N= 426 cancer N=58 SCT Median (range) 3.9 (0-18) y	M: 25(OH)D Chemiluminescent (Diasorin) assay T: Median (range) interval between diagnosis and 25(OH)D test 12.26 (2.05-36.4)y	RC	Race Pubertal status Age at diagnosis Gender Type of cancer diagnosis Endocrinopathy Radiation Glucocorticoid	25(OH)D insufficiency <20ng/ml Guidelines Institute of Medicine (IOM)	Correlations (*p*< 0.05) - Race (*p*< 0.001) (White: OR= 1.00; Black: OR=3.11; Others (Hispanic and Asian): OR= 2.08) - Pubertal status (*p*< 0.001) (Tanner 1-2: OR= 1; Tanner 3-5: OR= 2.29) - Age at diagnosis (OR=1.36; *p*= 0.01) No correlations: - Gender - Cancer diagnosis - Endocrinopathy - Radiation - Glucocorticoid
(Halton et al. 1995a) Canada	Moderate	ALL	N=40 Median (range) 3.9 (0.3-	M: High-pressure liquid chromatography D_3 and radiochemical assays (Fraher et al 1983)	CS	Age at diagnosis Leukocyte count Morphologic and immunologic type	$25(OH)D_3$ reference data (Fraher et al. 1983) $1,25(OH)_2D_3$ reference data	ALL (r= NR)

			17)y	T: at diagnosis		of leukaemia ALL	(Fraher et al. 1983):	
(Modan-Moses et al. 2012) Israel	Moderate	Malignancy	N=142 Median (range) 12.03 (1.1-5.8)y	M: DiaSorin LLAISON competitive two-step chemiluminescent immunoassay (DiaSorin, Stillwater, MN) T: one measurement at clinic during or soon after the completion of treatment	CS	Gender Age at diagnosis Cancer diagnosis Length of time from diagnosis Glucocorticoids SCT Seasonal variation Sun exposure Sun protection Anthropometry	25(OH)D status (reference data Misra et al. 2008)	Correlations: - Sun exposure (r= 0.195; *p*= 0.02; multilevel analysis: OR= -0.31; *p*= 0.001) - Sun protection (r= NR; p= NR; multilevel analysis: OR= 1.387; *p*= 0.002) - Younger age (r= -0.24; *p*= 0.004) Seasonal variation: 25(OH)D status was significantly lower in Winter compared to Summer (*p*= 0.001)
(Rosen et al. 2013) USA	Moderate	Benign tumor Malignancy Treated with combination of surgery, CT and/or RT	N= 201 Mean (±SD) at diagnosis 5.6(±0.3)y Mean (±SD) at initial visit 5.4 (±0.2)y	M: Liquid chromatography/tandem mass spectrometry (LC-MS/MS) by Sonora Quest Laboratories (Quest Nichols, san Juan Capistrano, CA) T: 2 assessments 1st at study entry (2 years after completion of therapy) 2nd follow-up	PC	Gender Ethnicity BMI Seasonal variation Cancer diagnosis Length of treatment	25(OH)D status (reference The Endocrine Society Holick et al 2011): Deficiency: <20ng/ml Insufficiency: 20-30ng/ml Sufficient: >30ng/ml	Correlations: - Younger age associated with higher 25(OH)D levels (r= -0.15; *p*= 0.03) - Cancer diagnosis (r= NR; *p*=0.017) Cancer diagnosis: osteosarcoma, retinoblastoma, hepatoblastoma and myeloid leukemias exhibited the lowest 25(OH)D concentrations
(Sinha et al. 2011) UK	Moderate	Pediatric cancer	Cases N= 61 Median (range) 11.1 (1.5-24.4)y	M DiaSorin 25-hydroxyvitamin D radio-immunoassay (RIA) (Cat #68100E Stillwater, Minnesota)	CC	Age Sex Ethnicity Cancer diagnosis History of travel abroad	25(OH)D Reference data (Weng et al. 2005, Vieth et al 2007, Bischoff et al 2006)	Correlations: - Cancer diagnosis (r= NR; *p*= 0.012 - Seasonal variation (r= NR; *p*=0.001) - Dark skin (r= NR; *p*= 0.001)

	Controls Median (range) 8.4(0.2-18.0)y	T: Once median (range) time post diagnosis 3.0(0.4-9.0) y *Some patients were on treatment and some had finished*	Treatment Seasonal variation	Deficiency : <20 ng/ml	- Younger age associated with higher 25(OH)D status (r= NR; *p*= 0.011)

D: Diagnosis; ALL: acute lymphoblastic leukaemia; NHL: non-Hodgkin's Lymphoma. Type of study: PC: prospective cohort study; CC: case-control study; CS: cross-sectional; NRCT: non-randomised clinical trial; RC: Retrospective cohort study; Treatment: CT: chemotherapy; RT: radiotherapy; SCT: stem cell transplant; ALT: aminotransferase; y: years; NR: not reported; NA: non-applicable or not part of study aims.

2.2.3.4 Quality of evidence

According to the assessment of quality of evidence using CASP methods, the evidence for the prevalence of vitamin D insufficiency and/or deficiency was highly variable with six (32%) studies being of weak quality, eleven (58%) of moderate quality and two (10.5%) studies were scored as "strong". The main issues identified with studies scored as weak and moderate were:

(i) It was difficult to establish the precision of the results due the absence of confidence intervals and/or relative risk (n=11).

(ii) Confounding factors present in the design and/or analysis of the study (n=9)

(iii) Lack of definition of outcome measures and normal vitamin D reference values (n=6).

Despite this, all selected studies that reported prevalence were considered to draw some general conclusions.

2.2.4 Discussion

This is the first systematic review appraising international evidence of the prevalence of vitamin D inadequacy in paediatric cancer patients and its possible causes. As only 19 studies of mainly small sample sizes and highly variable in quality were identified, at present there is insufficient robust evidence to accurately determine clear prevalence rates of vitamin D deficiency and insufficiency in the pediatric oncology population. Moreover, owing to the considerable diversity of the variables investigated and the lack of results reported in most studies, a meta-analysis including only studies investigating associations between age and vitamin D status was perfomed. Nonetheless, this systematic review importantly raises the possibility of a high prevalence of vitamin D deficiency and insufficiency in older children diagnosed with cancer, which may worsen during treatment and persist beyond completion of therapy.

2.2.4.1 Prevalence of vitamin D deficiency and insufficiency

The reported prevalence of vitamin D inadequacy at different stages of the disease, excepting at the time of diagnosis, it was highly variable across all types of

childhood cancer. Also, the fact that no study assessed all three stages of the disease in the same population made very difficult to establish clear patterns of change. Additionally, variability existed between the different geographical locations; Europe, North America and the Middle East. No eligible studies were found from Central and South America, Africa and most of Asia. The majority of the studies considered vitamin D status in children and young people diagnosed with haematological malignancies and very few investigated those diagnosed with solid tumors, brain tumors and benign tumors. Crucially, it was found that the terms deficiency and insufficiency in the context of vitamin D prevalence were used indiscriminately and dietary intake was not controlled for in most studies.

In agreement with Simmons et al (Simmons et al. 2011), but contrary to Atkinson (Atkinson 2008), this review suggests that the prevalence of 25(OH)D inadequacy in children diagnosed with haematological malignancies (deficiency; median 24% and insufficiency; median 39%) is similar to the general paediatric population (14-49%) (Choudhary et al. 2013); also found with the brain and benign tumor group, as the median prevalence for deficiency was 16% and for insufficiency 52%. Children diagnosed with solid tumors had a higher prevalence of 25(OH)D deficiency (median 61.5%) and insufficiency (median 71%) than those children diagnosed with other type of malignancies; consistent with one large study (n=2198) of adult oncology patients, which reported a prevalence of 25(OH)D deficiency of 75% (Vashi et al. 2010). Nevertheless, a few cautionary notes should be mentioned; the small number of studies looking at these population (Frisk et al. 2012, Bilariki et al. 2010, El-Ziny et al. 2007) and their small sample sizes, the different assay methods used to identify 25(OH)D status (Zerwekh 2008) and the geographical differences in the prevalence of 25(OH)D status.

Stratification of the studies by geographical location revealed some interesting differences; prevalence of 25(OH)D deficiency and insufficiency was highest in Europe (41% and 59% respectively), then the Middle East (24% and 51% respectively) and then North America (15% and 46% respectively). Moreover, whilst these values were higher than in the general paediatric population in Europe and the Middle East, they were not in North America. Of note, there was great variability

within the American data (prevalence ranging from 0% to 68.5%); all studies from Canada (Atkinson et al. 1998, Atkinson et al. 1989, Halton et al. 1995a) reported 0% prevalence of vitamin D deficiency and/or insufficiency, whilst those published in the USA ranged from 3% (Henderson et al. 1998) to 68.5% (Rosen et al. 2013). This could be attributed to the differences in vitamin D fortification polices from each country. In the USA fortification is discretionary (Institute of Medicine. Food and Nutrition Board 2010, Janz and Pearson 2013), whilst in Canada fortification of milk, margarine and plant-based milk is mandatory (Janz and Pearson 2013). An important observation is the difference in the prevalence of vitamin D inadequacy in both Europe and North America between studies from the 1980s and 1990s (Atkinson et al. 1998, Halton et al. 1996, Henderson et al. 1998, Atkinson et al. 1989, de Schepper et al. 1994) and those published after 2000 (Frisk et al. 2012, Choudhary et al. 2013, Bilariki et al. 2010, Rosen et al. 2013, Sinha et al. 2011); with most studies published after 2000 reporting higher prevalence of 25(OH)D than those published before. This could be a reflection of either an increase in 25(OH)D inadequacy (deficiency and insufficiency) in the paediatric oncology population, a recent increase seen in the general paediatric population (Ahmed et al. 2011, Holick 2006) or as previously suggested (Zerwekh 2008), the new more specific assay methods, which are more sensitive to vitamin D metabolites, thus providing more accurate results.

Seven studies (Atkinson 2008, Arikoski et al. 1999a, Bilariki et al. 2010, Halton et al. 1996, Henderson et al. 1998, Arikoski et al. 1999b, de Schepper et al. 1994, Halton et al. 1995a) reported 1,25(OH)D concentration and of these only one (de Schepper et al. 1994) did not measured 25(OH)D as well as 1,25(OH)D. As expected, there was a lot of variability in the reported 1,25(OH)D concentrations and no relationship between 25(OH)D and 1,25(OH)D (table 3.1). This may be explained by the vitamin D metabolism, as it is now acknowledged that 1,25(OH)D is tightly regulated by plasma calcium, instead of by the human vitamin D stores, with levels increasing when plasma calcium is reduced. 1,25(OH)D plasma levels also change rapidly due to the 4 hours half-life of this metabolite. Conversely, 25(OH)D is an accurate parameter of vitamin D body stores for the following reasons: (i) plasma 25(OH)D levels are normally influenced by sunlight exposure and dietary intake; (ii)

the half-life of 25(OH)D is three weeks, representing stores, which have been obtained from dietary intake and sunlight over a long period of time; (iii) the synthesis of 25(OH)D by the liver is not significantly regulated, but dependent mainly on substrate concentration (Holick et al. 2011, Holick 2009, Holick 2007, Zerwekh 2008).

Studies considering both plasma 25(OH)D and 1,25(OH)D levels alongside PTH were very heterogeneous in their findings, suggesting that in pediatric cancer patients there might be other factors influencing their relationship. At present, these are unknown, however it could be attributed to the following: type of cancer and the different forms of treatment, including chemotherapy and corticosteroid, which affects calcium homeostasis and can also cause hepatotoxicity as well as nephrotoxicity; thus interfering with the 25(OH)D, 1,25(OH)D and PTH metabolism (Atkinson 2008, Zhou et al. 2006).

2.2.4.2 Vitamin D supplementation

At present, there are current paediatric guidelines for supplementation of vitamin D (RCPCH 2013, Holick et al. 2011). However, this review did not find other studies meeting the eligibility criteria and also, investigating the changes in vitamin D status of paediatric oncology patients before and after supplementation, and assessing whether current guidelines are suitable for this population during and after treatment. Of note, a recent randomised double-blind placebo-controlled trial that did not meet our inclusion criteria has been published (Kaste et al. 2014). This study found no difference on lumbar spine bone mineral density in survivors of ALL following two years of either cholecalciferol and calcium supplementation or placebo (Kaste et al. 2014). Due to the limited body of evidence, more research that assesses the effects of vitamin D supplementation during and after treatment in this population is warranted.

2.2.4.3 Possible causes of vitamin D deficiency and insufficiency

There is a paucity of evidence examining possible causes of 25(OH)D inadequacy in paediatric cancer patients and the few published studies produced equivocal findings and considered different variables making it impossible to draw meaningful conclusions. Nonetheless, the meta-analysis performed showed that 25(OH)D

inadequacy, as in the healthy paediatric population (Holick 2007, El-Rassi and Baliki, G and Fulheihan El-Hajj, G), may be associated with older age (Choudhary et al. 2013, Rosen et al. 2013, Modan-Moses et al. 2012). Darker skin was also associated with lower plasma 25(OH)D levels (Choudhary et al. 2013, Sinha et al. 2011). A reduction in sunlight exposure is expected of this population if they adhere to the recommendations. As previously discussed, some cytotoxic drugs, radiotherapy treatment and the use of antifungals cause phototoxicity (Duncan et al. 2011), whilst those who have haematopoietic stem cell transplantations have an increased risk of developing skin malignancies if exposed to sunlight (Simmons et al. 2011). However, this was assessed in only two studies of moderate quality and small (n=61) and large (n=142) sample sizes (Modan-Moses et al. 2012, Sinha et al. 2011). Notably, the use of glucocorticoids showed mixed results; one large study (n=428) included in our review did not find associations between glucocorticoid use in children and adolescents diagnosed with an ICCC-3 cancer, treated with either chemotherapy or haematopoietic stem cell transplant and, decreased vitamin D levels (Choudhary et al. 2013); supported by another study not included in our review (Simmons et al. 2011). In contrast, a small study (n=48) of paediatric patients diagnosed with an ICCC-3 cancer included in this review showed correlation between glucocorticoid use and reduced 25(OH)D status (Arikoski et al. 1999b). Chemotherapy was not associated with increased 25(OH)D inadequacy (Arikoski et al. 1999b, Sinha et al. 2011); however both studies were small and were not stratified according to diagnostic criteria or chemotherapy. There were also mixed results with regards to associations between the different type of cancers and poor 25(OH)D status and only two studies specified the type of cancers that was found to correlate with low 25(OH)D, which was ALL (Rosen et al. 2013, Halton et al. 1995a) and the following: osteosarcoma, retinoblastoma, hepatoblastoma and myeloid leukaemias (Sinha et al. 2011). Finally, no study assessed treatment induced side-effects; including, hepatotoxicity and whether this had an effect on DBP concentrations and consequently on plasma 25(OH)D, kidney injury and mucositis, as possible causes of poor 25(OH)D status.

Taking into consideration the results of this systematic review and the limited number of studies available to date, this review propose for future research (table 2.16).

Table 2-16 Research recommendations to consider for the future

Research recommendations to consider for the future:

- Different forms of cancers need to be thought of as different diseases requiring very different treatments, as such should be presented separately.
- To assess vitamin D status, 25(OH)D (instead of 1,25(OH)D) should be measured
- Measurements of 25(OH)D need to be monitored at different stages of the disease and treatment course.
- Studies should specify the reference values of vitamin D to which they compare their data
- 25(OH)D deficiency and insufficiency should be clearly defined according to standard cut-off values.
- The following details need to be clearly specified: the timings of when the measurements are taken, clinical and demographic data, dietary intake, exposure to sunlight and specific treatment and treatment induced side-effects.

2.2.4.4 Quality of body of evidence, strengths and limitations of systematic review

Several limitations were identified in this review. Following the risk of bias assessment using the CASP tool, it became clear that the quality of studies varied considerably, which goes some way to explaining the great differences in reported prevalence of vitamin D deficiency and insufficiency. Potential bias might have occurred as this review excluded studies in which individuals younger and also older than 18 years of age were included in the same study and where data were analysed altogether. In spite of these limitations, the strength of this systematic review lies in its methodology. A comprehensive search of five electronic databases without language restrictions was conducted, experts in the field were contacted and the reference list of all the included studies was screened. Still, potential eligible studies

might have missed. Finally, the three variables included in the meta-analysis were not exactly identical as two were age at diagnosis and one was older age.

2.2.5 Conclusion

This systematic review represents the first attempt, to date, to review the epidemiological evidence for the prevalence of vitamin D deficiency and insufficiency in pediatric cancer patients worldwide, and to explore possible causes of vitamin D inadequacy. Unfortunately, these aims have been largely frustrated by a small number of eligible studies, the absence of a universal approach to measuring and defining 25(OH)D status, the failure of researchers to treat different types of cancer as different diseases and the fact that different research groups investigated different variables. Nonetheless, this systematic review highlights the following: the possibility of a high prevalence of vitamin D deficiency and insufficiency in paediatric cancer patients, which might also be associated with older age and darker skin; the need for future high-quality population based longitudinal cohort studies and high-quality clinical trials that assess the effect of vitamin D supplementation.

CHAPTER III

3 ASSESSMENT OF RELIABILITY, VALIDITY AND PRECISION OF ANTHROPOMETRICAL MEASUREMENTS PERFORMED IN HEALTHY CHILDREN

3.1 INTRODUCTION

It is essential to ensure that anthropometric measurements performed as part of research are as accurate, reliable, precise and as valid as possible (Atkinson and Nevill 1999, Ulijaszek and Kerr 1999, Jamaiyah et al. 2010). The prospective study performed to assess the determinants of nutritional risk in the paediatric oncology patients required regular anthropometric measurements, which were performed in two phases, phase-I and phase-II, by two researchers. The anthropometric methods being used included; weight, height or length (<2 years of age), middle upper arm circumference (MUAC) and triceps skin fold (TSF). In order to reduce random errors of measurements anthropometrists should follow standard protocols, have appropriate training and the equipment should be calibrated according to manufacturer instructions. However, even following expert training anthropometric measurements, especially MUAC and TSF, are subject to some degree of error within and between observers (Jamaiyah et al. 2010), thus these must be established to be able to interpret the data.

There are various terms that describe the degree of anthropometric measurement error: (1) precision, (2) accuracy, (3) validity and (4) reliability: (1) The degree of precision is defined as "the variability of repeated measurements due to intra- and inter observer error" (Ulijaszek and Kerr 1999) and it can be calculated by quantifying the technical error of measurement (TEM), which is the standard deviation of measurements taken independently from the same subject. Intra-observer TEM is the Standard Deviation (SD) of measurements taken by the same anthropometrist, whilst inter-observer TEM is the SD of the different measurements taken by two or more anthropometrists (Norton and Olds 1996). (2) Accuracy is the degree of closeness from a measurement taken to the true value of that measurement. As opposed to accuracy, inaccuracy is defined as systematic bias that results from the faults in the equipment or errors from the observer's technique (Ulijaszek and Kerr 1999). (3) Validity is defined as the extent to which a measurement is relevant with the desired characteristic/variable actually measured and, it is assessed along with reliability (Ulijaszek and Kerr 1999). (4) Reliability in this context is "the extent to which within subject variability is due to factors other than measurement error

variance (accuracy) and physiological variation (reproducibility)" (Ulijaszek and Kerr 1999). Reliability is calculated by assessing the intraclass correlation coefficient (ICC) and it is generally calculated from the results of analysis of variance (ANOVA) where 0 is not reliable at all and 1 is highly reliable (Atkinson and Nevill 1998, Norton and Olds 1999, Ulijaszek and Kerr 1999, Jamaiyah et al 2010).

In order to establish the precision and reliability of the measurements across the two phases, this study attempted to assess inter- and intra-observer TEM and ICC of weight, height, MUAC and TSF in a group of children.

3.1.2 Aims and Objectives

The main aim of this study was to assess the precision and reliability of the anthropometric measurements used by the two observers involved in the ENRICC study across phase-I and phase-II.

The objectives were:

- To calculate intra- and inter-observer TEM and ICC for the following anthropometric measurements: weight, height, MUAC and TSF.

3.2 METHODS

3.2.1 Study design and study population

A cross sectional study was performed. A minimum of 10 children including both boys and girls should be incorporated in the study for an optimal calculation of ICC and TEM (Ulijaszek and Kerr 1999). Therefore, the aim for the study population was to recruit a total of 10 children and young people through parents/carers via the QMU moderator email system. Additionally, TEM and ICC must be established in a population as similar as possible to the one to be tested in the principle study (Norton and Olds 1996). Therefore, the eligibility criteria were any healthy child and/or young people between the ages of 2 and 18 years and, the exclusion criteria included children younger than 2 and older than 18 years. Although children under the age of

2 were included in the ENRICC study, no suitable equipment was available to measure this in QMU.

3.2.2 Recruitment

This study was granted with ethical approval from the QMU Ethics Committee (appendix II) and volunteers were recruited through the QMU moderator email system. The study was explained to the parents and children who indicated an interest. Age-appropriate information was given to the families; children aged 6 to 11 were assigned the children's information sheet and those aged older than 11 years, the adult's information sheet (appendix II). Parents and children were allowed some time to consider participation and to ask questions prior consent was given. Participation in the study took place only if both the parent and the child agreed to participate. Oral and written consent was sought from both the parent and child. Each child was assigned an identification number to ensure anonymity.

3.2.3 Measurements

Age, gender and ethnicity were recorded from each volunteer. All anthropometric measurements were performed using standard procedures for which both researchers had been trained. The following anthropometric measurements were taken: weight, height, MUAC and TSF in a private location accompanied by the parents/cares and using the same standard protocols as in phase-I and phase-II of the project. As recommended by the literature (Atkinson and Nevill 1999, Ulijaszek and Kerr 1999) each child had four series of measurements taken (two by each anthropometrist) and three measurements taken at each series. This method has been shown to reduce bias as it minimises the possibility of memorising the measurements.

Weight was taken without shoes and heavy clothing using a standing SECA digital weight scale. Weight was measured to the nearest 0.1Kg. Height was measured using a wall mounted stadiometer (SECA Hamburg, Germany) to the nearest 0.1cm. Children were asked to stand with their heels and back in contact with an upright wall and, whilst the head was hold by the researchers to make sure the child was looking straight forward. Before the right-angle block was slide down to touch the head, children were asked to breathe in and out.

The MUAC of the non-dominant arm was measured with a measuring tape to the nearest 0.1cm. The volunteers were asked to stand, remove clothing that covered the non-dominant arm and bent their arm in a 90º position. Then the midpoint between the acromion (top of the shoulder) and the olecranon (tip of the elbow) was marked. Finally the circumference was measured from the marked point with the arm left loose (Frisancho 1974). The TSF was then taken using the same point reference than the one used for the MUAC with the arm also left loose. A Harpenden Skinfold caliper was used to assess TSF. The method used consisted of pulling gently with the thumb and the forefinger a fold of skin from the marked level (midpoint) with the skinfold parallel to the long axis of the arm. Then the jaws of the caliper were placed at the marked level, which were left for 10 seconds to allow for an optimum reading (Oakley et al. 1977). The caliper measured to the nearest 0.2mm.

A low TEM should be achieved to obtain accurate measurements (Pederson and Gore 1996). Reference levels for intra- and inter-observer TEM have been proposed for three accreditation anthropometrists' level, which are presented in table 3.1. Reliability was calculated using ICC which measured the correlation coefficient. The reliability ranges from 0-1 where 0 is not reliable, 0-0.2 is slightly reliable, 0-2-0.4 is fairly reliable, 0.6-0.8 is substantial and 0.8-1 is almost perfect reliability (Pederson and Gore 1996, Ulijaszek and Kerr 1999). An ICC larger than 0.95 should be achieved for reliable measurements (Ulijaszek and Kerr 1999).

Table 3-1 Proposed target for intra- and inter-observer TEMs for the three accreditation levels following the training course (Gore et al 1996)

		Level 1	**Level 2**	**Level 3**
Intra-observer	Skinfolds	10.0	7.5	7.5
	Other measurements	2.5	2.0	2.0
Inter-observer	Skinfolds	12.5	10.0	10.0
	Other measurements	2.0	1.5	1.5

Note: level 2 and 3 are the same

3.2.4 Statistical Analysis

The intra-observer TEM and %TEM for the two measurements were calculated by firstly performing a one way ANOVA test. This test provided the error mean square, which was then used in the following equations to calculate both TEM and %TEM (Ulijaszek and Kerr 1999): TEM =√error mean square and % TEM = (TEM/mean) x 100. The inter-observer TEM was calculated by using the following equation (Ulijaszek and Kerr 1999):

$$TEM = \frac{\sqrt{((\Sigma^{n}(\Sigma^{n} M^{2})-(\Sigma^{n} M^{2})/K))}}{(k-1)}$$

K= number of observers

N= number of subjects

M= measurement

ICC was calculated using a one way random single measures test on SPSS®.

3.3 RESULTS

Ten children were recruited between March and July 2012. Of these 50% (n=5) were males and 50% (n=5) were females. The median (IQR) age was 8.5 (6.5-10.7) years and all of them were Caucasian. Intra- and inter-observer TEM, %TEM and ICC were calculated for all measurements (tables 3.2, 3.3, 3.4), as there was not a statistically significant difference between the measurement's means of either each observer and between observers.

Table 3-2 TEM and ICC (observer I)

	Height (cm)	Weight (Kg)	MUAC (cm)	TSF (mm)
F*	0.00	0.00	0.00	0.02
***p* value**	1.00	0.99	0.99	0.97
TEM cm	0.00	0.02	0.12	0.17
TEM±68% CI	0.00	0.02±0.03	0.12±0.17	0.17±0.24
TEM±95% CI	0.00	0.02±0.05	0.12±0.33	0.17±0.48
TEM%	0.06	0.02	0.60	1.30
ICC	0.99	0.99	0.96	0.96

*F: Difference between the means obtained from each series of measurements

Table 3-3 TEM and ICC (observer II)

	Height (cm)	Weight (Kg)	MUAC (cm)	TSF (mm)
F*	0.00	0.00	-0.05	-0.11
***p* value**	0.99	0.99	0.97	0.97
TEM cm	0.00	0.00	0.11	0.13
TEM±68% CI	0.00	0.00	0.11±0.15	0.13±0.18
TEM±95% CI	0.00	0.00	0.11±0.3	0.11±0.36
TEM%	0.01	0.01	0.22	1.23
ICC	0.99	0.99	0.98	0.96

*F: Difference between the means obtained from each series of measurements

Table 3-4 Inter-TEM and ICC (observer I v observer II)

	Height (cm)	Weight (Kg)	MUAC (cm)	TSF (mm)
F*	0.10	-0.10	-0.41	-0.85
***p* value**	0.99	0.99	0.83	0.70
TEM cm	0.79	0.47	2.78	2.83
TEM±68% CI	0.79±1.12	0.47±0.66	2.78±3.93	2.83±4.00
TEM±95% CI	0.79±2.23	0.47±1.32	2.78±7.86	2.83±8.00
TEM%	0.83	0.68	1.67	1.69
ICC	0.99	0.99	0.96	0.96

*F: Difference between the means obtained from each series of measurements

3.4 DISCUSSION

The intra and inter-observer TEM for all measurements, apart from inter-observer TEM for MUAC, was below the proposed accreditation for level 2 post-course anthropometrists (Gore et al 1996). However, the inter-observer TEM for MUAC was 2.78cm, which is slightly higher than the proposed accreditation for level 1 post-course anthropometrist (2.5cm) (Gore et al 1996). These results indicate that the intra-observer TEM for weight, height, MUAC and TSF and the inter-observer TEM for weight, height and TSF were minimal. Thus, it is reasonable to accept that these measurements have reduced systematic bias and are consequently accurate (Ulijaszek and Kerr 1999). In contrast, more systematic bias between observer 1 and observer 2 are luckily to occur following MUAC measurements, which agrees with published

studies reporting that higher TEM are generally obtained when measuring MUAC and TSF (Ulijaszek and Kerr 1999, Jamaiyah et al. 2010, Geeta et al. 2009, Ulijaszek and Lourie 1997).

The intra- and inter-observer ICC for all measurements were ≥ 0.95 indicating a high level of reliability (Pederson and Gore 1996, Ulijaszek and Kerr 1999). Although the inter-observer TEM for MUAC was slightly higher than the proposed accreditation for the level 1 anthropometrists, the reliability (ICC) was 0.96 indicating also a high level of reliability.

3.5 CONCLUSION

In conclusion, the measurements obtained by the two researchers showed a high level of precision, with a small technical error for all measurements, apart from MUAC, and a high level of reliability for all measurements. Consequently, it is acceptable to assume that the two researchers were able to detect anthropometric changes with a high degree of precision and reliability, and that measurements of MUAC will be considered with caution when interpreting the results from the longitudinal study. Both the TEM and ICC will be considered in chapter IV, discussion section.

CHAPTER IV

4. PROSPECTIVE STUDY: THE DETERMINANTS OF NUTRITIONAL RISK IN PAEDIATRIC CANCER PATIENTS

4.1 AIMS AND OBJECTIVES

The aims of this study were:

(i) to investigate the prevalence of malnutrition (undernutrition, overnutrition and obesity) of paediatric cancer patients;

(ii) to identify patterns of change in the nutritional status of paediatric cancer patients throughout the course of the disease and treatment;

(iii) to determine potential factors which may contribute to the development of malnutrition in newly diagnosed paediatric cancer patients;

(iv) to establish whether nutritional status at diagnosis is associated with clinical outcomes

(v) to identify the use of nutritional support and whether patients at high risk of undernutrition were treated with any type of nutritional therapy.

The objectives of this study included:

- To quantitatively describe demographic, clinical and nutritional characteristics of this population.
- To identify patterns of change by prospectively monitoring nutritional status parameters, which include measurements of growth, body composition and vitamins and minerals, from the time of diagnosis until the end of the study period (3 years).
- To prospectively monitor newly diagnosed paediatric cancer patients for a maximum period of 3 years to identify short-term and medium-term determinants that might predict the development of undernutrition and overnutrition.
- To prospectively monitor plasma vitamins and minerals status to identify and explore potential factors that may contribute to abnormalities.
- To prospectively monitor the use of nutritional support in the different type of cancers and to identify how many PEM and undernourished (established by BMI) patients were receiving nutritional support.

- To establish whether undernutrition and obesity at the time of diagnosis are associated with a negative event, which includes relapse, becoming palliative or mortality during the 3 years of the study period

4.2 METHODS

4.2.1 Study design and time line

This was a prospective cohort study originally designed in 2007 by Edinburgh Nutritional Risk in Childhood Cancer (ENRICC) and with the initiation of data collection in August 2010. The project can be divided into two phases: phase-I and phase-II: Phase-I was commenced in August 2010 were data were collected until January 2012 and phase-II, which was started in February 2012 and completed in April 2014.

In this study repeated measurements were performed every three months from the time of recruitment over a period of one year and every six months thereafter. Individuals recruited in phase-I were followed until the end of phase-II for a maximum period of three years and those recruited in January 2014 were followed for a minimum period of three months (figure 4.1).

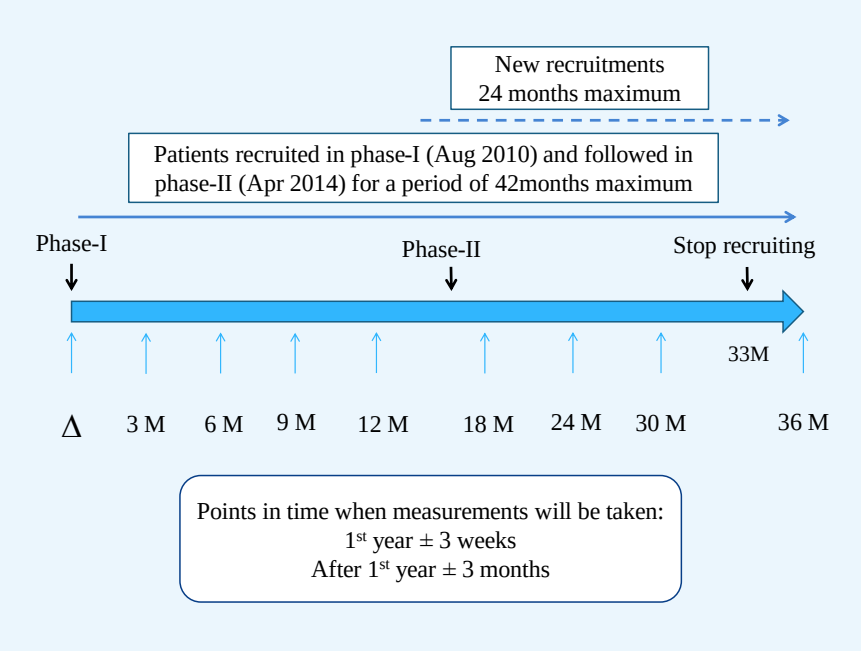

Table 4-1 Representation of the longitudinal cohort study; phase-I and phase-II

4.2.2 Study population

Eligibility criteria of the study population included:

- Children and young people under the age of 18 years who were diagnosed with either a cancer included in the ICCC-3 or with Multisystem Langerham's Cell Histiocytosis (LCH)
- Children diagnosed between August 2010 and January 2014 in SE Scotland and treated in the RHSC, Edinburgh and/or Ninewells Hospital, Dundee.
- Children who were treated with chemotherapy and/or radiotherapy or combinations therapies, which could include either chemotherapy or radiotherapy with or without corticosteroids, proton therapy and/or surgery.

The following children were excluded from the study:

- Children who were treated palliatively from the time of diagnosis
- Children who were diagnosed with a second cancer and as a consequence had previously been treated.
- Children who became palliative during the study period were withdrawn from the study.
- Children treated with surgery only.

The controls used in this study were patients who met the eligibility criteria but did not take part in this study. This was done to establish whether the cohort (participants) were representative of the paediatric oncology population from SE and E Scotland.

4.2.3 Recruitment

Children and their families (or carers) were provided with written and oral information once a confirmed diagnosis of cancer and a plan for treatment was established by the Haematology/Oncology team (appendix III). Families were given a minimum of 24 hours to read the information and think about participating in the study. Only then written consent was obtained from the older children, parents or carers (appendix III) and assent from children. All families were informed that they

were not under any obligation to participate and that they could withdraw at any time if they wished to.

4.2.4 Demographics and clinical parameters

Demographics and clinical data were collected from medical notes. As a proxy marker for socioeconomic deprivation level of individuals, this study used individual residence postcodes to assess deprivation level of areas of residence using the Standard Index of Multiple Deprivation (SIMD) (The Scottish Government 2012). SIMD is presented as a quintile where "I" denotes the most deprived and "V" the least deprived.

The following clinical data were collected: cancer diagnosis, date of diagnosis and age at diagnosis; from which decimal age was then calculated at every time point. The type of cancer was then classified according to ICCC-3 (Steliarova-Foucher et al. 2005) (appendix I) to be able to compare with other paediatric cancer populations and for statistical purposes; to determine the nutritional risk of this population. In addition, the following data were recorded: length of treatment, treatment induced side effects, death, and event-free survival as well as whether the patients became palliative. The variables were stratified into two groups: "events" and "event free survival" (EFS), which in cancer is defined as responding to treatment without the following events: relapse, death, metastasis or becoming palliative during treatment.

The treatment was recorded in two different ways: treatment protocol and whether the child received chemotherapy, radiotherapy and proton therapy, and also whether the child had had any surgery. Any changes in treatment protocols or other treatment modalities were also noted during the data collection period. Intensity of treatment is often related to the severity of disease; as such treatment was divided into three groups: high risk, medium risk and low risk to establish possible associations between treatment intensity and nutritional risk (appendix I) (Kazak et al. 2012). Those children who started on low/medium risk protocols but were soon after diagnosis (less than two months) changed to high risk protocols were classified as high risk. High dose corticosteroids are used in alternative phases for prolonge periods of time in all ALL treatment protocols, and small doses are also used as anti-emetics or to reduce intracranial pressure in patients with brain tumours (Davis et al.

2004). Although, evidence have showed that their use may lead to obesity (Davis et al. 2004, Dulloo et al. 2012, Reilly et al. 2001), this study did not consider their use in isolation owing to the prospective nature of this project. Instead they were considered as part of the whole therapy.

4.2.5 Measurements of growth and body composition

Measurements of weight and height at the time of diagnosis, prior to recruitment, were obtained from clinical notes as volunteers were generally recruited a few weeks into treatment. Weight and height measurements were obtained following standard procedures for infants and children (Tanner 1990b) and using the available equipment from outpatient's clinics or from the hospital's wards.

Children under the age of 2 had their length and weight measured. Length was measured between two (one researcher and either a member of staff or a parent) on a supine position lying down on a flat surface provided by a standard length scale (SECA 399, Hamburg Germany) and completely naked. Whilst the child lied down, one person held the head in an upward position and another straightened the legs with one hand and placed the sliding board in contact with the child's heels with the other hand, making sure that the toes were facing upwards. Infants of less than one year were weighted nude using an electronic baby scale (SECA 232, Hamburg, Germany) providing an estimated weight to the nearest 0.1g.

Children older than 2 had their standing height measured without shoes using a wall mounting stadiometer which measured height to the nearest 0.1mm (SECA Hamburg, Germany). Children were asked to stand with their heels and back in contact with an upright wall and, whilst the head was hold by the researchers to make sure the child was looking straight forward. Before the right-angle block was slide down to touch the head, children were asked to breathe in and out. Weight was taken without shoes and heavy clothing using a standing scale (SECA 599, Hamburg, Germany), which estimated weight to the nearest 0.1Kg.

The radiale-stylion segment length (ulna length) was measured in one patient who was not able to stand up due to muscular dystrophy, using Harpenden Holtain Anthropometer. The measurements were taken by placing one arm of the caliper on

the radiale and the other arm on the stylon landmark according to Norton and Tim's (1996). The caliper estimates the length to the nearest 0.1mm over a range of 50mm to 570mm. Height was then estimated with the following equation height = 4.459 x (length) + 1.315 x age + 31.485 (Gauld et al. 2004). Ulna length was chosen instead of other limbs because evidence showed to be reproducible and to predict height more precisely in school-age children. It also appears to be superior in patients with neuromuscular weakness and spinal deformities (Gauld et al. 2004).

BMI expressed as Kg/m^2 was then calculated using the standard equation weight divided by squared height. Finally, height, weight, head circumference and BMI centiles and standard deviations were calculated using LMS Growth programme (Harlow Healthcare, UK).

Measurements of arm anthropometry, MUAC and TSF were taken using standard techniques as it has been described in chapter III, section 3.1.3.4. The raw measurements were then used to calculate UAMA and UAFA by using an equation proposed by Frisancho (1981).

$$A\ (mm^2) = л/\ 4 \times d^2$$

$$M\ (mm^2) = (C - л\ T)^2/\ 4л$$

$$F\ (mm^2) = A - M$$

A: Upper arm area; M: UAMA; F: UAFA; C: MUAC mm; T: TSF

BIA was measured using a SF-BIA Quantum II RJL System at a frequency of 50 KHz and the device was calibrated according to manufacturer's instructions (once per month). The measurements were also performed using manufacturer instructions. Children were asked to lie down on their backs with the arms adopting a 45° position and their legs slightly separated. All four electrodes were placed on the right side of the body, unless contraindicated for clinical reasons. Two electrodes were placed on one hand and two on one ankle as shown in figure 4.1. For most reliable readings, it is recommended that individuals have their bladders empty, are not on intravenous fluids or treatment at the time of the reading and, infants should have their wet nappies removed. This was done whenever possible. The estimation of FM and FFM

was calculated using the TBW equation developed by Schaefer et al (1994, 2000). This was originally developed in 112 healthy children and adolescents (age 3.9-18) and then validated in a group of children diagnosed with chronic renal failure who were also on haemo or peritoneal dialysis (n=23) (Schaefer et al. 1994a, Schaefer et al. 2000). Like paediatric cancer patients, children with chronic renal failure also experience rapid shifts in body fluids and many are treated with corticosteroids (Schaefer et al. 1994a, Schaefer et al. 2000). Furthermore, the authors reported a coefficient of variation of 8.5%, which is significantly smaller than those reported in other equations developed from similar populations (Wühl et al. 1996). Thus, the TBW equation developed by Schaefer et al. (1994, 2000) seemed more appropriate than the one specifically developed for paediatric cancer patients (Brennan 1998), which reported large limits of agreement and it has not been validated yet.

$$TBW = 0.65\ (h^2/I) + 0.68 \times age\ (years) + 0.15$$

$$FM\% = W - TBW$$

$$FFM\% = FFM \times BW\ /100$$

TBW: total body water; h: height; I: impedance; W: weight

All measurements were taken three times whenever possible and a mean of the three values was calculated. When the three measurements were impossible to obtained, the mean of the two or a single one was used for data analysis.

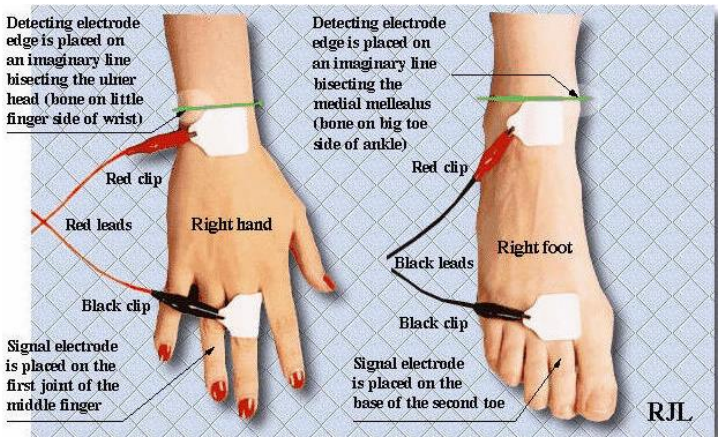

Figure 4.1 Position of the electrodes for measurements of Bioelectrical Impedance Analysis

4.2.6 Blood collection and analysis of samples

Blood samples were collected by the nursing or medical staff either when the children had all the other measurements taken or at the next routine blood test. Due to ethics blood was not extracted for research purposes only, but taken in conjunction with routine clinical blood tests. Blood samples were immediately sent to the RHSC laboratory where they were processed and then analysed or sent somewhere else for analysis. Bloods taken to assess full blood count, liver function and urea and electrolytes, as well as renal function were all analysed in the Edinburgh RHSC laboratory facilities. Standard sensitivity C-reactive protein (ss-CRP) was analysed in the Royal Infirmary of Edinburgh using the Abbott Architect C8000 analyser by a turbidimetric method (see appendix IV).

Full nutritional screening included the assessment of vitamin and minerals. The vitamins measured were: vitamin B12, folate, and the fat soluble vitamins A, D and vitamin E/Cholesterol (E/Ch) and, the minerals measured included: ferritin (for iron stores), zinc, selenium, copper, magnesium and calcium. Bloods obtained for full nutritional screening were analysed in the Royal Infirmary laboratory of Glasgow (see appendix IV). All analyses were performed using standard operating procedures by the hospital's own accredited laboratory. Methods of specific vitamins and minerals with scientific interest will be specified. Vitamins B12, folate and ferritin were all measured using a chemiluminescent immunoassays performed on the Abbott Architect i2000 Analyser. Vitamins A and E/Ch were measured by high performance liquid chromatography (HPLC) with UV detection. Trace metals (zinc, copper and selenium) were measured by Inductively Coupled Plasma Mass Spectrometry.

4.2.7 Assessment of dietary intake and energy requirements

In order to determine children's macronutrient and micronutrient intakes and any pattern of change throughout the study period, dietary intake was obtained at every time point by using a 24 hour multi-pass recall method. The researcher interviewed the patients and/or the parents by asking to recall everything that the child had drank and eaten the day before, if that was a typical day. In cases where it was not a typical day due to treatment procedures such as fasting, families were asked to recall a typical day representative of that time. For completeness, further questions about

portion sizes, cooking methods and brands, as well as whether the child had had specific foods from different food groups were asked. As patients on treatment for cancer often experience appetite and taste changes, questions about this were also asked.

To assess the prevalence of patients needing nutritional support, the reasons for nutritional support and the type of nutritional treatment prescribed, all this information was recorded. The nutritional intervention was classified following NICE 2006 guidelines:

- Oral nutrition support (ONS)
- Enteral tube feeding (ETF): nasogastric (NG), nasojejunal (NJ), gastrostomy (PEG), jejunostomy (PEG-J) tube feeding
- Parenteral nutrition (PN)
- Advanced nutritional support (ETF and PN)

Both macronutrient (fat, protein and carbohydrates) and relevant micronutrient [vitamins (A, B12, C, D, E and folate) and minerals (calcium, copper, iron, magnesium, selenium and zinc)] intakes were analysed using WinDiets® (Univation Ltd 2005) programme. Estimated energy intakes from the 24 hour multi-pass analysis were then compared to energy requirements to assess whether the children were meeting requirements. If the child was on nutritional support the total energy intake was calculated altogether. The Scientific Advisory Committee on Nutrition (SACN) published in 2011 and based on updated evidence recommends the use of the Henry equation (2005) to estimate energy intake in infants, children and adolescents. Thus this equation was used for the purpose of this study. As discussed in chapter I, section 1.3, there is no evidence supporting an increase in REE in this population as a result of an increased in metabolic response. Additionally, this population have an increased risk of becoming obese and there is evidence reporting sedentary lifestyle in this population (Brinksma et al. 2014, Reilly 2009a). Thus no stress factor was added to the equation. Instead a physical activity level (PAL) of low physical activity (10^{th} centile) adjusted for growth was added: 0-3 years: 1.3; 3-10 years: 1.35 and 10-18 years: 1.58, which was advised in the SACN (2011) document.

4.2.8 Reference values

The reference data to assess growth was obtained from height, weight and BMI standards published by Cole et al. (1995), which were specifically developed from UK children from the 1990s (Cole et al. 1995a). New growth charts for children under the age of 4, which combined WHO standards with UK preterm and birth data (32-42 weeks) from the 1990s, were introduced at the end of 2010. These were not used as reference data in order to keep continuity from phase-I into phase-II of the study as the standards used here were adopted in 2007. Additionally, this standard is most commonly used for research purposes making the results more suitable for comparison with published studies.

BMI definitions of malnutrition were obtained from the Department of Health (2012) and SACN as this provides with official UK national definitions. Underweight was defined as BMI $\leq 2.3^{th}$ centile (-2 SD), overweight between $\geq 85^{th}$ ($\geq$1.05 SD) and $<95^{th}$ (<1.63 SD) centile and obese $\geq 95^{th}$ centile ($\geq$1.63 SD). The prevalence of malnutrition in the paediatric oncology population was then compared with UK national data obtained from the Nutrition and Diet National Survey (NDNS) (Department of Health 2012). New data on prevalence of malnutrition in British children has been published, however this was only published in September 2014, once data analyses had already been performed (Bates et al. 2014).

The reference centiles used for MUAC and TSF and, UAMA and UAFA were Frisancho (1974) and Frisancho (1981). There are other published references including: WHO (2011), Oguz et al. (1999), Oakley et al. (1977), Ozturk et al. (2009); however they all carry disadvantages with them including small sample size (Oguz et al. 1999), populations that are not representative of the Scottish paediatric oncology patients (Ozturk et al. 2009) and reference designed for infants aged less than 42 weeks. Therefore the reference values from Frisancho (1974 and 1981) were used for many reasons. Firstly, the sample population from Frisancho's reference data were thought to be more representative of the Scottish paediatric oncology population than that of the WHO (2011). Frisancho's references were developed from a large sample from the USA; population similar to that of Scotland, whilst reference from WHO (2011) were developed from developed and developing

countries. Also, WHO (2011) does not have UAMA and UAFA centiles, whilst Frisancho's references does (1974 and 1981). All references are adjusted for age and gender and MUAC, TSF, UAMA and UAFA's definitions of malnutrition were: undernutrition $\leq 5^{th}$ centile (Frisancho 1974, Garofalo et al. 2005; Oguz et al. 1999; Smith et al. 1991), overnutrition between $\geq 85^{th}$ and $<95^{th}$ centile and obesity $\geq 95^{th}$ centile (Frisancho 1974 and Frisancho 1981). These generated categorical data, which were then normalised according to age and gender by calculating the percentage of the 50^{th} centile. Thus, allowing for statistical calculations and comparisons.

FM% and FFM% values were obtained from BIA measurements. At present, there is no published reference representing the age range 0-18 years. Thus two were selected for the purpose of this study: Fomon et al. 1982 for children under the age of 10 and Wells et al. 2012 for children between 10-18 years of age. All references are matched for age and gender. The reference values published by Fomon et al 1982 have been obtained from a 4 compartment model, which includes %FM and %FFM. The percentage of body weight from the FFM is divided into 4 further compartments: protein, TBW (extracellular and intracellular fluid), minerals (osseous and non-osseous) and carbohydrates. As the BIA only allows to obtain measurements of 3 compartments (FM, FFM and TBW), all the compartments included in the FFM were summed. This value was then used to estimate the 50^{th} centile of FFM.

The reference ranges used for blood parameters were those published by NHS Scotland Laboratory Handbook, which are all adjusted for age.

UK macronutrient intake recommendations for infants, children and adolescents are only available for protein, thus the reference nutrient intake (RNI) for protein were used to compare with the protein intake from this cohort (Department of Health 1991). Additionally, fat and carbohydrate recommendations for infants (< than 1 year) have not been described yet, thus the average proportions of energy yielding nutrients of infant formula were used, which are 50% (~30g per day) for fat, and 40% of TEI (65-70g per day) for carbohydrates (Wojcik et al. 2009). The reasons for using this reference are firstly, it has been recommended by the UK DRVs due to the

low protein content of breast milk, which is described to be insufficient for formula fed infants (Department of Health 1991). Secondly, dietary intake for infants in this cohort of patients is only available for those who were formula fed or were on nutritional support as opposed to breast fed babies. Therefore, these values appeared more suitable for this population. For the percentage of macronutrient intakes for children older than 1 year, UK adult recommendations were used, which are 35% for fat and 50% for carbohydrates. The total requirements of carbohydrates and fats (in grams) were calculated from the total energy requirements and the percentage recommendations.

4.2.9 NHS ethical approval

This study was granted with NHS ethical approval from the NHS Scotland (NHS REC 06-51104-52) on the 1st of June 2007 (appendix V). The researcher also had the relevant enhanced disclosure and honorary contract, which allow carrying out the data collection of this study. All patients' data were anonymised and kept confidential.

4.2.10 Statistical analyses

Descriptive statistics were used to evaluate the prevalence of malnutrition (frequencies), changes in nutritional status according to growth and body composition measurements and blood parameters. All data were tested for normal distribution. Normally distributed data were presented as mean ± standard deviation (SD) and non-normally distributed data as median and interquartile range (IQR). Comparisons of different groups (diagnostic criteria, treatment risk and gender) were established by independent t-test, ANOVA (normally distributed data), Mann-Whitney and Kruskal Wallis (non-normally distributed data). Correlations between variables were tested using Pearson's (normally distributed data) and Spearman's correlation (non-parametric) and categorical data was tested for associations using the $\chi 2$ test. Participants (cohort) and non-participants (controls) were compared to establish whether this paediatric cancer cohort was representative of SE and East Scotland. Results were expressed using 95% confidence intervals, odds ratios and $p < 0.05$ was considered statistically significant.

Multilevel analyses were performed to establish the following; (i) statistical changes in nutritional status assessed by using growth and body composition over time and (ii) possible factors that may contribute to changes in both growth and body composition over time.

To establish changes in nutritional status over time a multilevel growth model was used. Prior to perform this analysis all groups (diagnostic criteria and treatment risk groups) were compared to establish whether the groups were significantly different. There was not statistically significant difference between the groups, therefore all cancer patients were analysed all together. The total length of time was 18 months as there was not enough data at any time point from 18 months until 36 months to perform growth multilevel analyses. Changes in nutritional status according to both growth and body composition was presented at three time points 0-3 months, 0-9 months and 0-18 months. This was decided due to the different trajectories each variable followed. The outcome variables analysed were divided into growth measurements and body composition. Growth measurements included: BMI centile, HFA centile, MUAC (percentage of 50^{th} centile) and TSF (percentage of 50^{th} centile) and body composition measurements included: FFM% and FM% obtained from both arm anthropometry parameters and BIA.

To establish factors that may contribute to changes in nutritional status at each time point (0-3 months, 0-9 months and 0-18 months) the mixed multilevel model was used. The outcome variables were kept as before (growth and body composition parameters) and the following factors tested were diagnostic criteria, treatment risk, age at diagnosis, whether the patient was on any nutritional support during the interval period and the total estimated energy intake also from the appropriate time interval. The different factors were tested one at the time and only those that reached a relaxed significance of 0.1 were included in the conditional model. Final significance was established at $p < 0.05$. All analyses were performed using The Statistical Package for Social Studies (IBM-SPSS for Windows Statistics, 19), whilst graphs were performed using IBM-SPSS 19, Excel and Graph Pad Prisma 5.

4.3 RESULTS

4.3.1 Patient's demographics

179 patients were diagnosed with childhood cancer between the 1st of August 2010 and the 31st of January 2014 in the Edinburgh Royal Hospital for Sick Children. Of these 78 (43%) did not meet the eligibility criteria for the reasons described in figure 4.2 and, a total of 101 (57%) were considered eligible. 19 (19%) patients refused to participate for the reasons described also in figure 4.2, thus a total of 82 (81%) patients were studied.

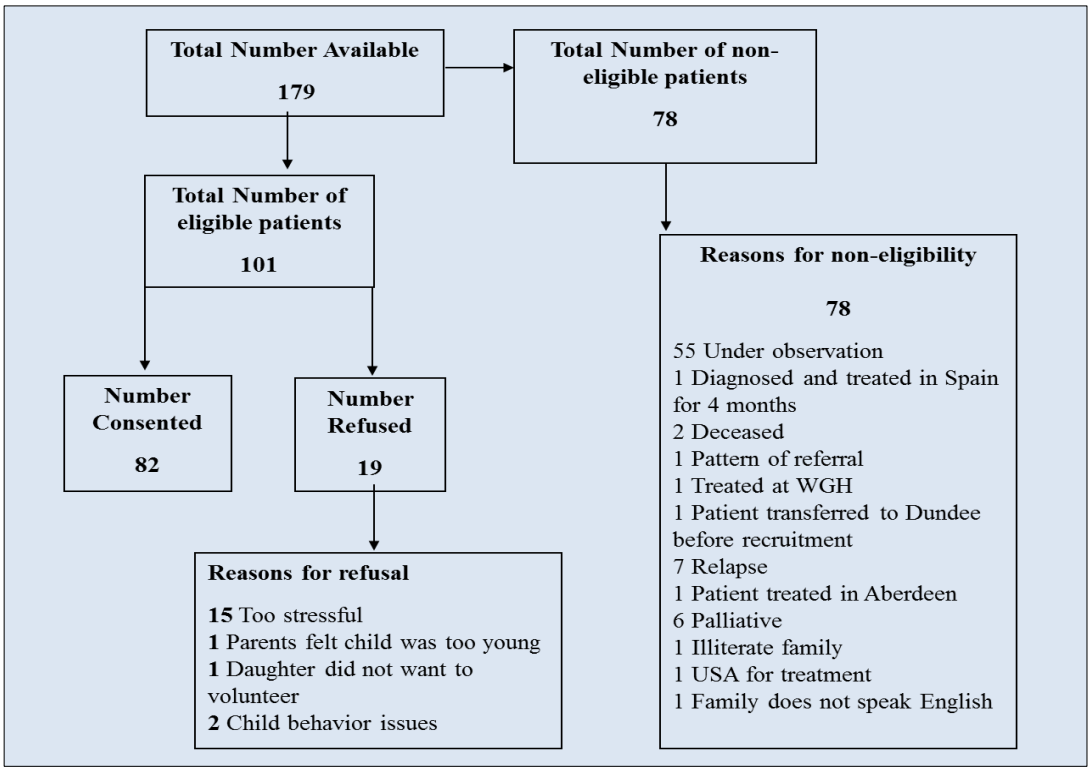

Figure 4.2 Flow diagram showing patient's accrual

Table 4.2 shows that this cohort of 82 patients was representative of the whole paediatric oncology population from SE Scotland. Childhood cancer incident rates according to ICCC-3 are presented in table 4.3. At the end of the data collection period (31st April 2014), the survival rate was 90% (n=74), the death rate was 10% (n=8) and the event free survival rate was 85% (n=70). Thus 15% (n=12) of patients had either relapsed; their cancer had metastasised or did not respond to treatment. Of these, 67% (8/12) died, 17% (2/12) became palliative, 17% (2/12) were receiving treatment by the end of the study and 8% (1/12) survived.

Table 4-2 Characteristics of the n=82 Paediatric Oncology cohort and n=22* controls (non-participants)

Patients' characteristics	**Cohort**			**Controls**			**P**
	Median	**IQR**	**95% CI**	**Median**	**IQR**	**95% CI**	
Age at diagnosis (years)	3.88	1.96-8.83	4.69-6.88	6.52	3.91-10.65	5.37-9.26	0.06[1]
		N	**%**		**N**	**%**	
Gender							0.5[2]
Male		46	56		10	45.5	
Female		36	44		12	54.5	
Diagnostic criteria							0.9[3]
ST		35	43		10	45.5	
HM		36	44		11	50	
BT		7	8.5		1	4.5	
OAD		4	5		0	5	
Deprivation**							0.6[2]
I		15	18		7	32	
II		12	15		2	9	
III		15	18		2	9	
IV		24	29		8	36	
V		15	18		3	14	
Ethnicity							0.5[3]
White		80	98		21	95.5	
Non-white		2	2.4		1	4.5	

*N=22: 19 (refused to participate) + 3 (met criteria but were not approached as advised by consultants); **Deprivation I-V where I denotes the most deprived and V the economically most advantageous families; [1]Mann-Whitney; [2]Chi square test; [3]Fisher's Exact Test

Table 4-3 Cancer diagnosis according to ICCC-3 of the n=82 Paediatric Oncology cohort and n=22* controls

Diagnosis	Cohort n=82		Control n=22	
	N	**%**	**N**	**%**
I-Leukaemias	35	43	11	50
ALL	29	35	11	50
AML	3	4	0	0
CML	2	2	0	0
HLH	1	1	0	0
II-Lymphoma	10	12	3	14
III-CNS tumours	5	6	2	9
IV-Neuroblastoma	6	7	2	9
V-Retinoblastoma	2	2	0	0
VI-Renal tumours	6	7	0	0
VII-Hepatic tumours	1	1	0	0
VIII-Malignant bone tumours	4	5	3	14
IX-Soft tissue sarcoma	5	6	1	4.5
X-Germ cell tumours	1	1	0	0
XI-Malignant epithelial neoplasm	4	5	0	0
XII-Others and unspecified malignant neoplasms	0	0	0	0
Other associated diagnoses	3	4	0	0
LCH**	3	4	0	0

*N=22: 19 (refused to participate) + 3 (met criteria but were not approached as advised by consultants); **LCH: Langerham's cell Histiocytosis.

A total of 24 treatment protocols were used to treat this cohort of patients. These were further stratified into different treatment risks and subsequently randomised into different arms of the trials. Therefore, for simplification of the data, the treatment type was presented into five categories: (i) 56% (n=46) were treated with chemotherapy only; (ii) 4% (n=3) with a combination of chemotherapy and radiotherapy; (iii) 18% (n=15) with a combination of chemotherapy and surgery; (iv) 19.5% (n=16) with chemotherapy, radiotherapy (or proton therapy) and surgery and,

(v) 4% (n=3) were treated with other forms of treatment like HSCT. Finally, the cohort was stratified according to risk of treatment failure (low risk, medium risk and high risk) to be able to perform correlation statistics. There were 22% (n=18) classified as low risk, 37% (n=30) as medium risk and 41.5% (n=34) as high risk.

The number of patients and measurements available at each time point is presented in table 4.4.

Table 4-4 Patient's accrual and follow up at each time point and number of patients having had each type of measurement taken

Time point	Patients availability	Drop outs*	BMI	MUAC	TSF	BIA	Dietary intake	Bloods
Recruitment	82	0	81	79	75	60	77	77
3 months	82	6	75	71	70	56	75	70
6 months	73	19	54	49	49	38	54	42
9 months	65	14	51	48	47	37	51	34
12 months	55	14	42	41	40	30	42	25
18 months	47	13	34	33	32	28	34	25
24 months	35	11	24	24	24	20	24	13
30 months	19	7	12	12	12	10	12	6
36 months	16	9	7	7	7	6	7	3

*Drop outs due to: deceased patients, palliative treatment, treatment given in centres other than RHSC, Edinburgh and Ninewells Hospital, Dundee and patients who missed appointments.

4.3.2 Prevalence of malnutrition and changes in nutritional status according to growth and body composition measurements

The prevalence of malnutrition in paediatric cancer patients was investigated at each time point from the time of diagnosis and recruitment (baseline) up to a maximum of 36 months and a minimum of 3 months. Prevalence and changes in nutritional status were presented all together (all cancers) and stratified by gender and cancer type

(solid tumours, haematological malignancies, brain tumours and other associated diagnoses).

4.3.2.1 Measurements of growth

(i) Prevalence of malnutrition

The prevalence of malnutrition (undernutrition, overnutrition and obesity) where all cancers are presented together varied at each time point and with each measurement. The prevalence of well nourished paediatric cancer patients according to BMI are presented in figure 4.3 for comparative reasons.

The prevalence of undernutrition was identified using BMI, MUAC and TSF measurements. At the time of diagnosis and baseline, the prevalence of undernutrition was higher than at any other time during the study period. This ranged between 13% and 15% depending on the type of measurement used. BMI (11/81) and MUAC (10/79) identified the same percentage of undernourished patients (13%), whilst TSF (11/75) identified a slightly higher percentage (15%). According to BMI, at 9, 12 and 18 months there were no undernourished patients; however MUAC and TSF identified between 3% and 6% of the total cohort of patients. Finally, at 30 and 36 months none of the patients were undernourished (figure 4.4).

The prevalence of overnutrition and obesity were established according to BMI and TSF. Both measurements identified similar prevalence of overnutrition at baseline, 3, 6, 18 and 24 months; however this differed from each other at 9, 12 and especially at 36 months (see figure 4.5). As it can be seen from the graph, the highest prevalence of overnutrition was at 30 months [BMI; 2/12 (17%)] and 36 months [TSF; 2/7 (29%)], whilst the lowest was at 24 months [BMI and TSF; 2/24 (8%)].

BMI identified consistently higher prevalence of obesity than TSF throughout the study period and there were no obese patients at 36 months. Figure 4.6 shows that obesity was least prevalent at diagnosis as BMI identified 15% (12/82) of patients and TSF only 1% (1/75); whilst obesity was most prevalent at 30 months with 33% of patients identified using BMI (4/12) and 25% TSF (3/12).

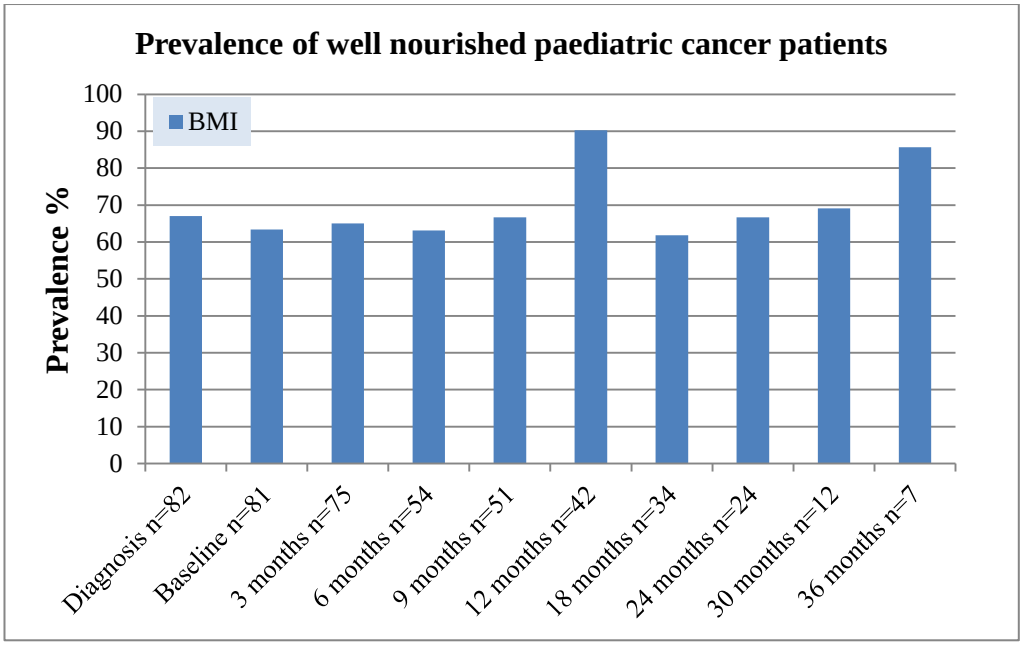

Figure 4.3 Prevalence of well nourished paediatric cancer patients at different stages of the disease and according to BMI centile between 2.3rd -84th centile.

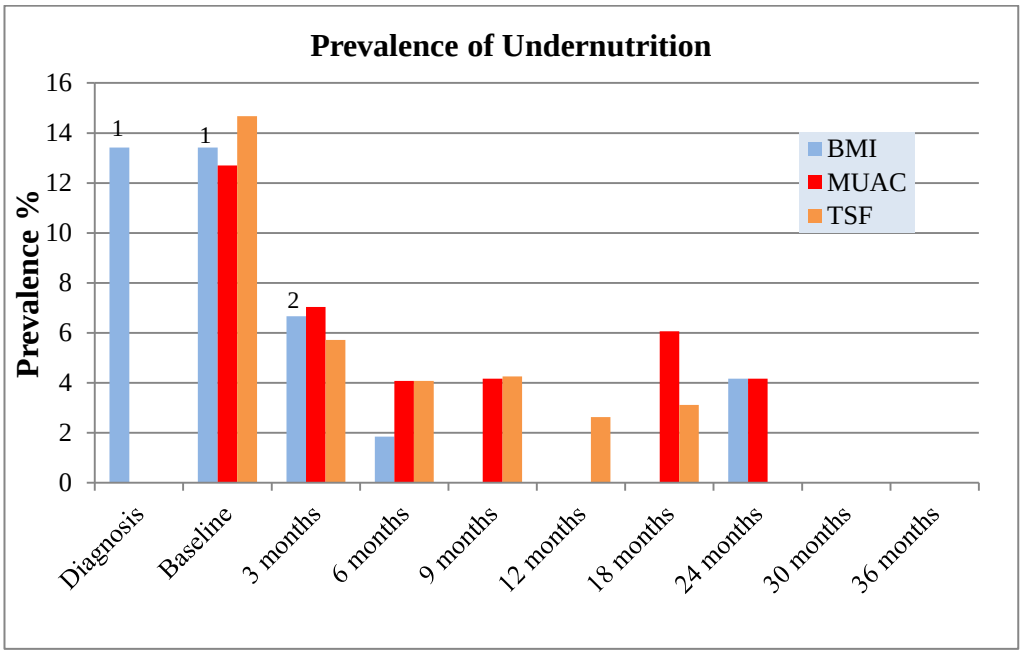

Figure 4.4 Prevalence of undernutrition at different stages of the disease and according to BMI (<2.3rd centile), MUAC (<5th centile) and TSF (<5th centile) in all cancers.

[1]χ^2 (9.65), p=0.03; [2] χ^2 (5.274), p=0.025 against UK prevalence of undernutrition (DH 2012). For BMI, MUAC and TSF n values at different time points see table 4.4.

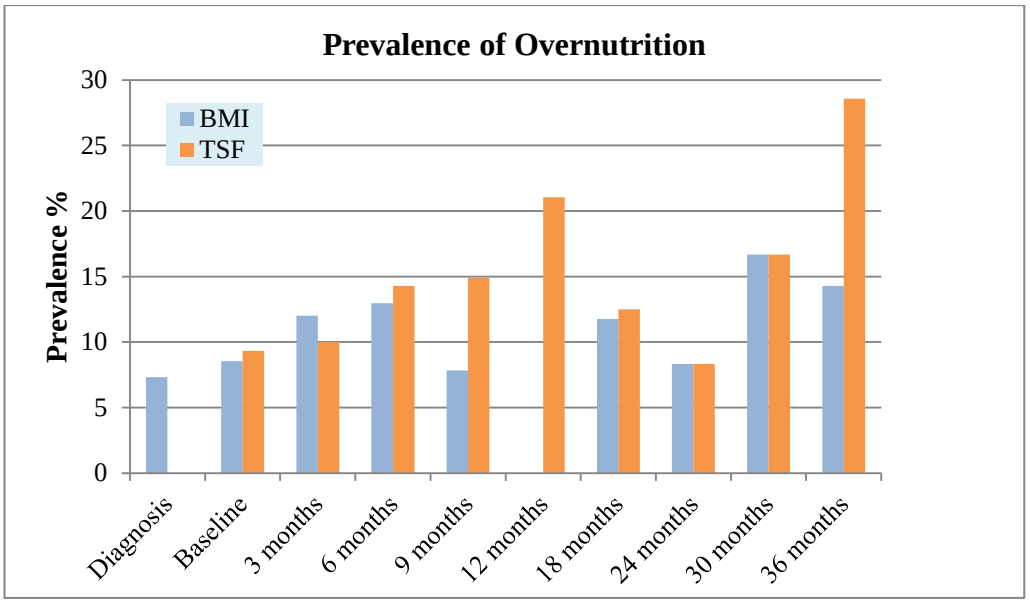

Figure 4.5 Prevalence of overnutrition at different stages of the disease and according to BMI (85-95th centile) and TSF (85-95th centile) in all cancers.

For BMI and TSF n values at different time points see table 4.4.

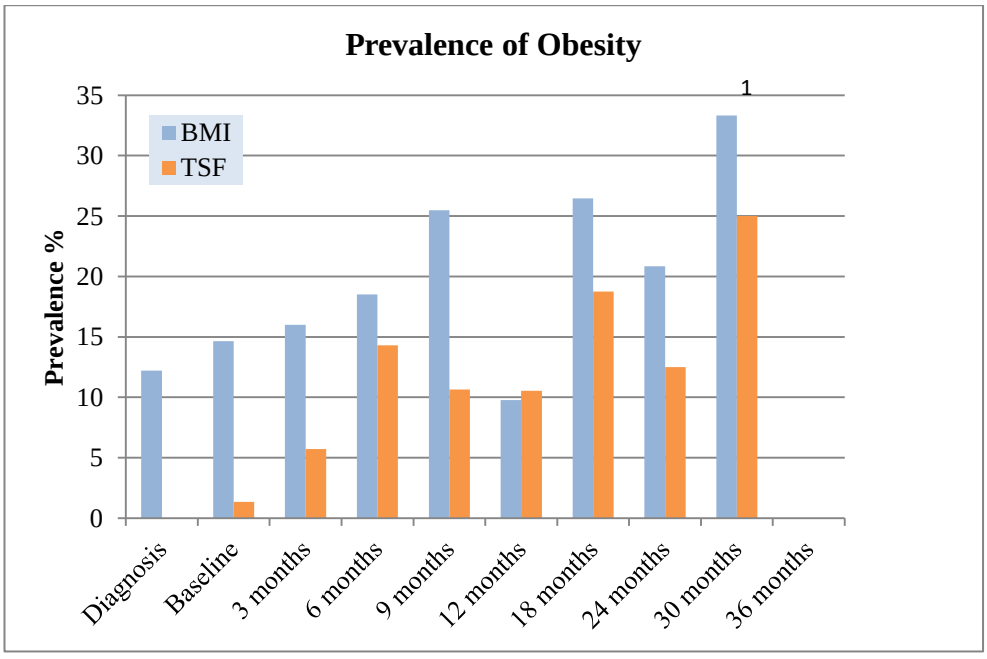

Figure 4.6 Prevalence of Obesity at different stages of the disease and according to BMI (>95th centile) and TSF (>95th centile) in all cancers.

[1]χ^2-test (5.274), p=0.03 against UK prevalence of obesity (DH 2012). For BMI and TSF n values at different time points see table 4.4.

Prevalence of malnutrition was also stratified by gender. A series of Mann Whitney test showed that there were not statistically significant differences between BMI centiles of males and females at any stage (p> 0.05). However, the odd ratios showed that both males and females diagnosed with cancer were 10 times more likely to be undernourished at the time of diagnosis and recruitment than healthy children from the UK (DH 2012). Males with a cancer diagnosis were 2.2, 2.27 and 4.28 times more likely to be overweight at 12, 30 and 36 months respectively than healthy males, whilst the likelihood of females with cancer to be overweight or obese did not differ from healthy female children. Finally, the odd ratio for males with cancer at 30 months was 3.57 higher than healthy male children (see figure 4.7).

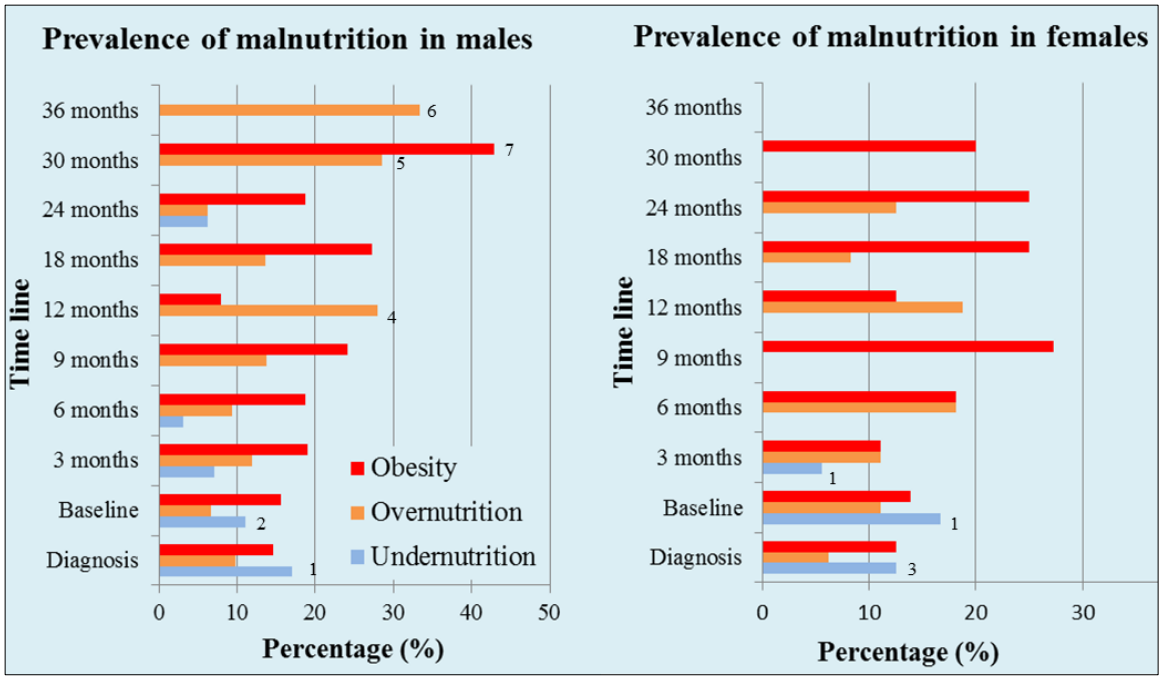

Figure 4.7 Prevalence of malnutrition according to BMI and stratified by gender at different stages of the disease and treatment

[1] χ^2 (13.085), p=0.001; [2] χ^2 (6.664), p<0.02; [3] χ^2 (8.6), p=0.005; [4] χ^2 (5.007), p<0.03; [5] χ^2 (5.711), p<0.03; [6] χ^2 (8.882), p<0.03; [7] χ^2 (14.742), p<0.001 against undernutrition, overnutrition and obesity UK prevalence (DH 2012)

Finally, prevalence of malnutrition was stratified by type of cancer; solid tumours, haematological malignancies, brain tumours and other associated diagnoses (OAD),

and type of measurement; BMI, MUAC and TSF. At baseline, the BMI centiles from the different diagnostic groups did not statistically differ (figure 4.8); however, prevalence of undernutrition measured by BMI, MUAC and TSF was highest in the solid tumour group: 17% (BMI), 22% (MUAC) and 18% (TSF). Children diagnosed with solid tumours [$\chi^2(1)$ 19.908, $p<0.001$; $\chi^2(1)$ 13.085, $p<0.001$], haematological malignancies [$\chi^2(1)$ 4.714, $p=0.03$; $\chi^2(1)$ 6.664, $p=0.01$] and brain tumours [$\chi^2(1)$ 13.085, $p<0.001$; $\chi^2(1)$ 9.783, $p=0.03$] had a significantly higher risk of being underweight than healthy children from the UK at the time of diagnosis and baseline. In contrast, children diagnosed with OAD and brain tumours showed the highest prevalence of overweight and obesity also at baseline; however this was not significant. Following 6, 9 and 12 months of treatment no patients diagnosed with a solid tumour was classified as undernourished; but 4% (BMI, MUAC and TSF) of those diagnosed with haematological malignancies and 33% (TSF) of brain tumours were undernourished at 6 months. These two groups also had the highest prevalence of overweight and obesity at 6, 9, 12 and 24 months (see table 4.4). Prevalence of overweight was significantly correlated with haematological malignancies [$\chi^2(1)$ 17.887, $p<0.001$] at 12 months, brain tumours [$\chi^2(1)$ 8.882, $p=0.005$] at 6, 9 and 24 months and OAD [$\chi^2(1)$ 8.882, $p=0.005$] at 12 months. Finally, although obesity prevalence were higher in all types of cancer (apart from solid tumours) than in the healthy UK children population (Department of Health 2012), only children with brain tumours had a significantly higher risk of becoming obese at 6 and 9 months [$\chi^2(1)$ 5.274, $p<0.03$].

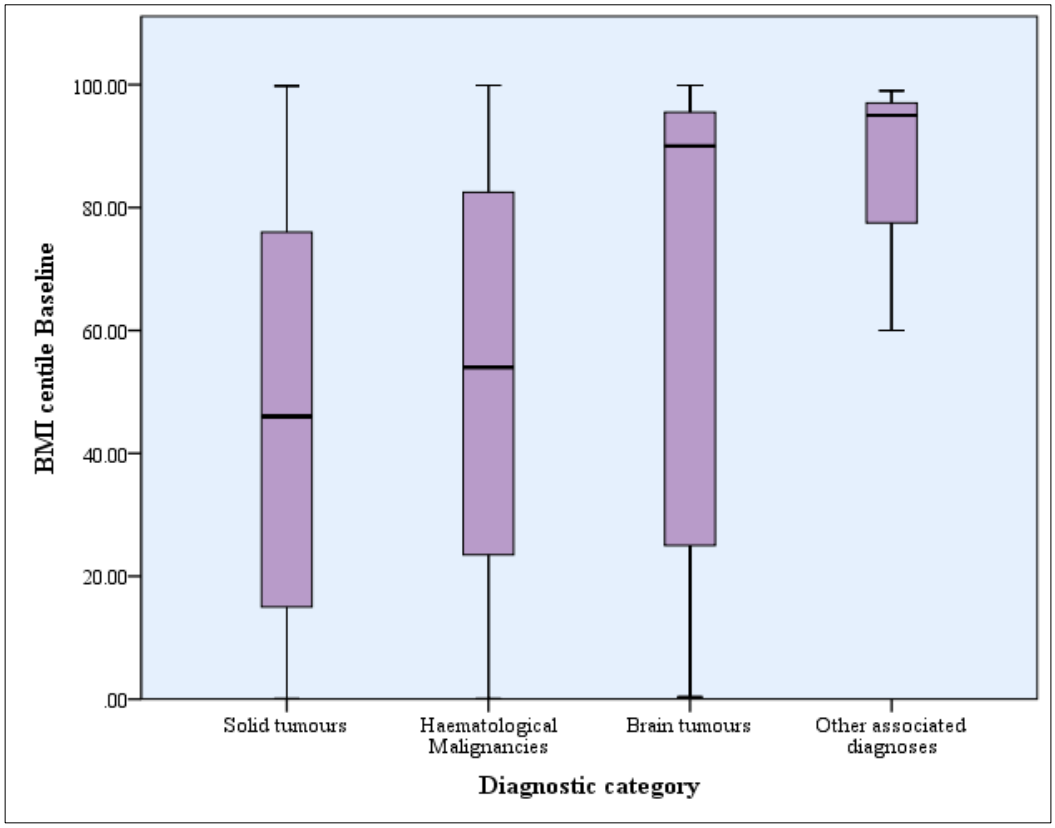

Figure 4.8 Baseline BMI centiles (median) stratified by diagnostic category.

Solid tumours: n=36; Haematological malignancies: n=35; Brain tumours: n=7; OAD: n=4

Overall MUAC and TSF identified more undernourished and overnourished children, whilst BMI identified higher obesity frequencies. Malnutrition measured as BMI is the primary outcome of the study, consequently the limit of agreement of MUAC and TSF were measured against BMI at the time of recruitment and 24 months post diagnosis. The Kappa limit test showed poor agreement between BMI and both MUAC [K 0.1, p=0.01) and TSF [K 0.3, p<0.001] at identifying nutritional status.

(ii) Changes in nutritional status

Changes in growth were assessed using BMI centiles, MUAC (percentage of the 50th centile) and TSF (percentage of the 50th centile) throughout the course of the study period. Changes in nutritional status assessed by BMI were explored in each individual and in all paediatric cancer patients. Data was then stratified according to

diagnostic criteria and treatment risk to establish whether there were any differences in patterns of change between the groups. Multilevel analyses were then performed to establish (i) statistical changes in growth parameters over time; (ii) whether the growth trajectory differed between the diagnostic groups and (iii) possible factors that contribute to changes in growth over time.

Figure 4.9 shows the high variability in BMI centiles of each individual at the time of diagnosis and throughout the course of treatment. It can also be seen that although most individuals were well-nourished at diagnosis, many changed to overnourished or obese during the study period. Overall, BMI centile in paediatric cancer patients increased throughout the course of treatment. The median (IQR) BMI centile at diagnosis was 50 (19-84.5) and at baseline was 49 (24-86). This increased steadily at every time point until it reached a peak at 18 months [median (IQR) 74 (48-96)] and 30 months [median (IQR) 85 (39-98)] (figure 4.10). It can be seen from figure 4.11 that children diagnosed with solid tumours had consistently lower BMI centiles than the haematological malignancy group. Additionally, by the end of the study period the median (IQR) BMI centile increased from 46 (17-78) at diagnosis to 65 (38.5-87.5) at 3 months in children with solid tumours and from 54 (22-83) at diagnosis to 95.5 (71.5-99) at 30 months in the haematological malignancy group. Children treated with high treatment risk protocols had the lowest BMI centiles during the initial phases of treatment, however this increased sharply by the end of the treatment (figure 4.12). Also, infants (age < 2 years) had lower BMI centiles during the initial phases of treatment than older childre and teenages during the whole study period (figure 4.13). For changes in MUAC, TSF and UAMA and UAFA see figures 4.13 and 4.14.

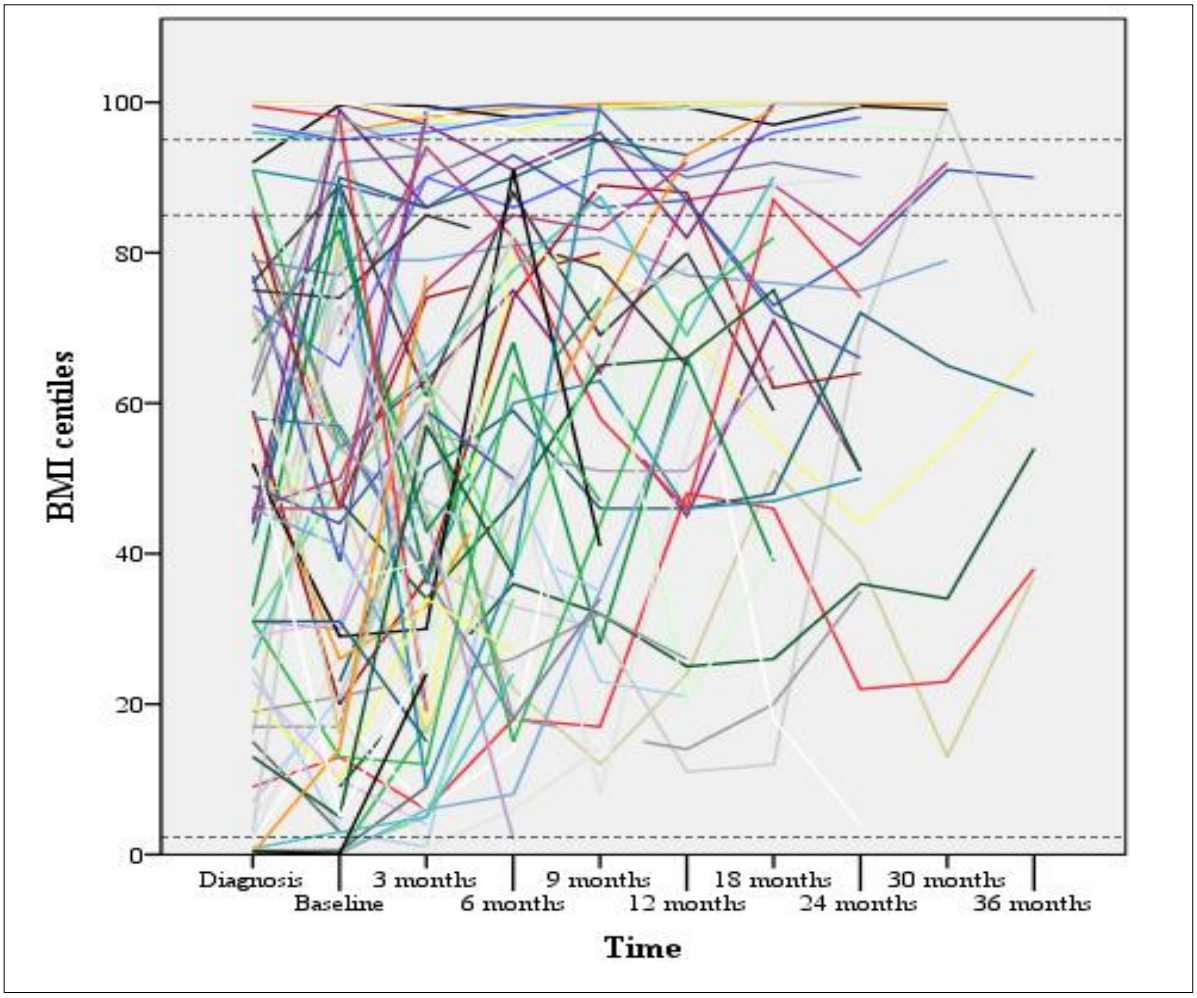

Figure 4.9 Individual changes of BMI centiles from the time of diagnosis up to 36 months

Horizontal lines indicate nutritional status: below bottom line (<2.3rd centile: undernutrition), above medium line (≥85th centile: overnourished) and above top line (≥95th centile: obese)

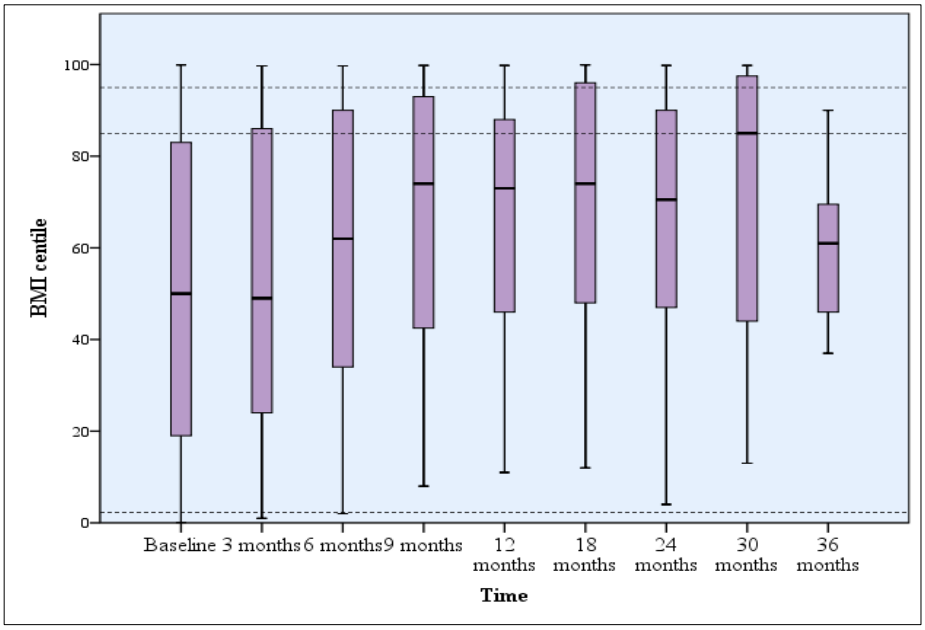

Figure 4.10 Changes in median BMI centiles in paediatric cancer patients from the time of diagnosis up to 36 months

Horizontal lines indicate nutritional status: below bottom line (<2.3rd centile: undernourished), above medium line (≥85th centile: overnourished) and above top line (≥95th centile: obese)

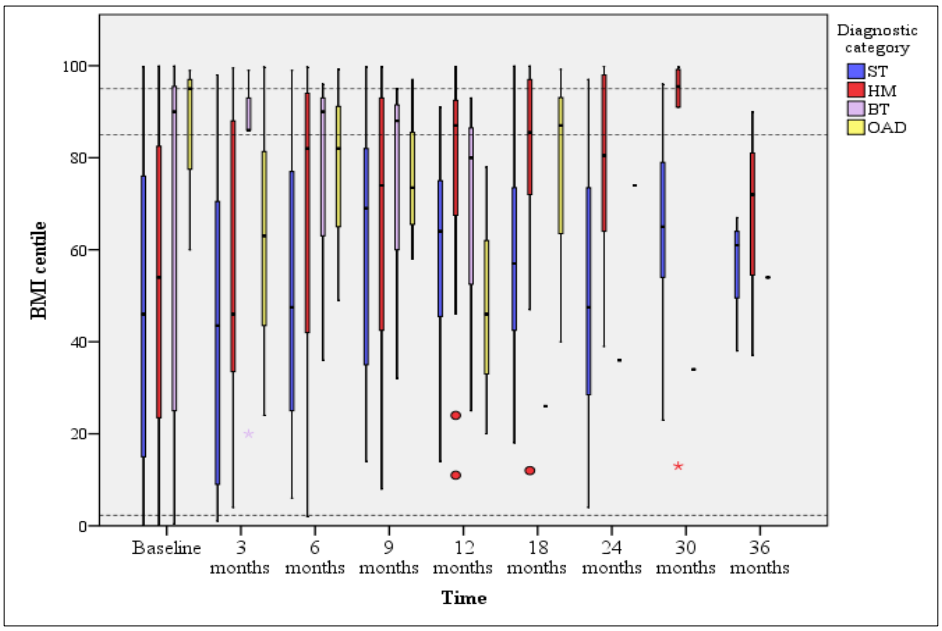

Figure 4.11 Changes in median BMI centiles in the different diagnostic groups from baseline up to 36 months

ST: solid tumours; HM: Haematological malignancies; BT: brain tumours and OAD: other associated diagnoses. Horizontal lines indicate nutritional status: below bottom line (<2.3rd centile: undernourished), above medium line (≥85th centile: overnourished) and above top line (≥95th centile: obese).

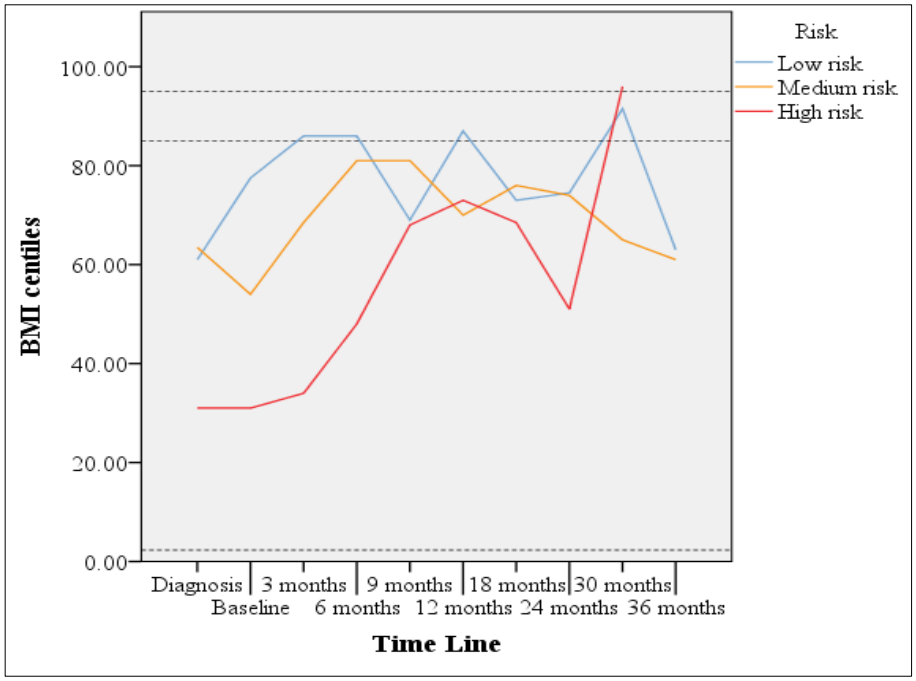

Figure 4.12 Changes in median BMI centiles with data stratified according to treatment risk from the time of diagnosis up to 36 months

Horizontal lines indicate nutritional status: below bottom line (<2.3rd centile: undernutrition), above medium line (≥85th centile: overnourished) and above top line (≥95th centile: obese).

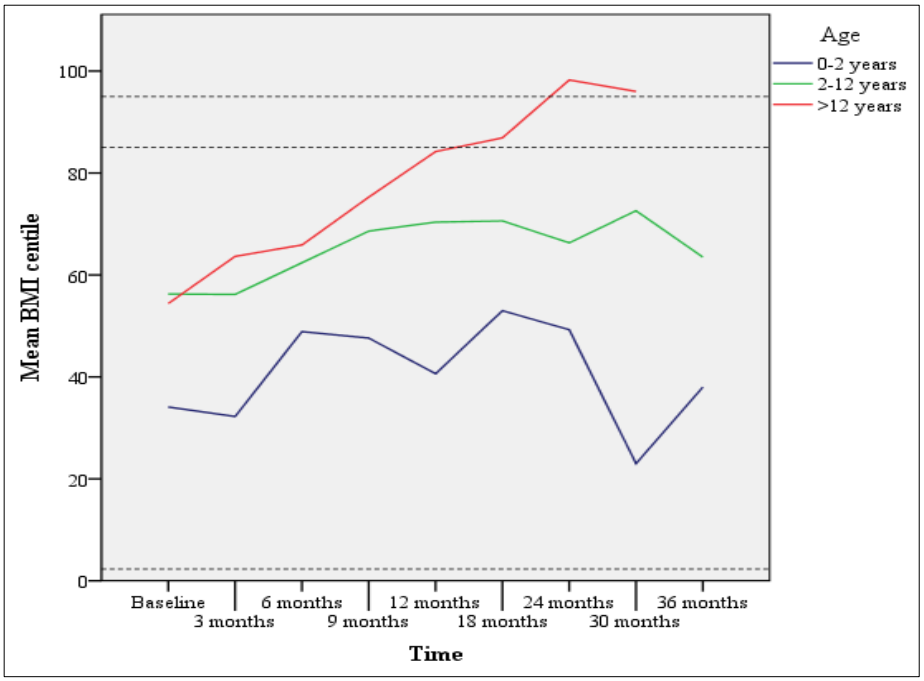

Figure 4.13 Changes in BMI centiles with data stratified by age groups

Horizontal lines indicate nutritional status: below bottom line (<2.3rd centile: undernutrition), above medium line (≥85th centile: overnourished) and above top line (≥95th centile: obese).

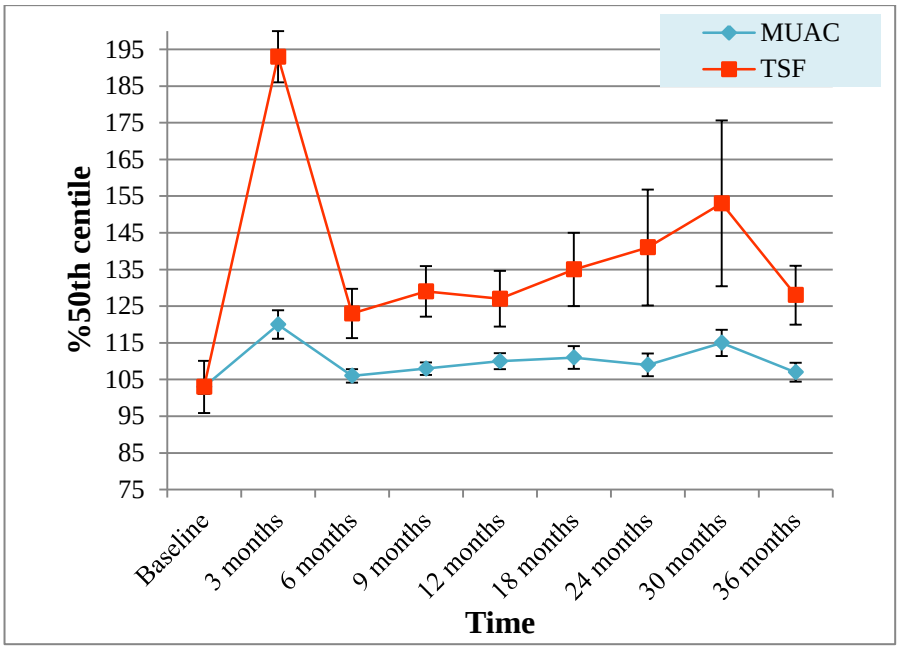

Figure 4.14 Changes in MUAC and TSF in all paediatric cancer patients from baseline up to 36 months

Data has been standardised by calculating the percentage of the 50th centile (Frisancho 1974). Error bars (±SEM). For MUAC and TSF n values at different time points see table 4.4.

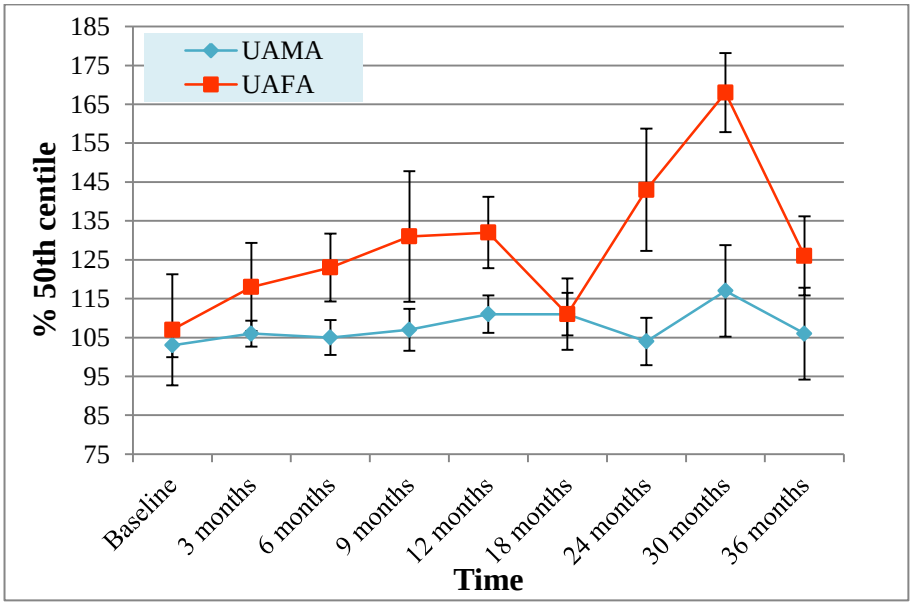

Figure 4.15 Changes in UAMA and UAFA in all paediatric cancer patients from baseline up to 36 months

Data has been standardised by calculating the percentage of the 50th centile (Frisancho 1981). Error bars (±SEM). For UAMA and UAFA n values at different time points see table 4.4.

Table 4-5 Prevalence of malnutrition according to BMI and TSF and stratified by type of cancer

Time Line	Nutritional status	Solid tumours				Haematological malignancies				Brain tumours				OAD[1]			
		BMI		TSF		BMI		TSF		BMI		TSF		BMI		TSF	
		%	N	%	N	%	N	%	N	%	N	%	N	%		%	N
Baseline	Undernutrition	17[2]	35	18	33	11[2]	36	12	33	14[2]	7	17	6	0	4	0	4
	Overnutrition	0		9		5.5		9		28.5		0		0		25	
	Obesity	8.5		0		14		0		28.5		17		50[2]		0	
6 months	Undernutrition	0	24	0	22	4	23	5	20	0	3	33	3	0	4	0	4
	Overnutrition	8.3		9		17		25		33[2]		0		0		0	
	Obesity	8.3		13		26		15		33[2]		33		25		0	
12 months	Undernutrition	0	16	0	16	0	19	0	17	0	3	0	3	-	0	33	3
	Overnutrition	6		19		42[2]		23.5		33[2]		33		-		0	
	Obesity	0		0		21		23.5		0				-		0	
24 months	Undernutrition	12.5[2]	8	0	14	0	14	0	14	-	0	-	0	-	0	-	0
	Overnutrition	0		14		14		7		-		-		-		-	
	Obesity	12.5		28.5		28.5		26		-		-		-		-	

[1]OAD: other associated diagnoses (N= 4; Langerham's Cell Histiocytosis); [2] χ^2-test; $p<0.05$ against undernutrition, overnutrition and obesity UK prevalence (DH 2012).

4.3.2.2 Body composition

Body composition, FFM and FM, was calculated from arm anthropometry (MUAC and TSF) and BIA. Measurements of arm anthropometry first generated data on UAMA and UAFA, from which centiles presented in a ratio-fashion are available. In order to assess the prevalence of PEM and undernutrition, overnutrition and obesity, the UAMA and UAFA centiles were used respectively for reference (Frisancho 1981). This categorical data was then normalised according to age and gender by calculating the percentage of the 50^{th} centile, which allowed for statistical comparison. FFM% and FM% obtained from arm anthropometry was then estimated following Frisancho's equation (1981). The reference values used for BIA (Fomon 1982 (children <5 years), Wells 2012 (children >5 years)) allowed calculating the percentage of the 50^{th} centile for both FFM and FM. And the equation by Schaefer (2000) was used to estimate FFM% and FM%.

Table 4-6 Comparison between children identified as PEM by UAMA and the nutritional status established by BMI

Time line	**UAMA**	**BMI**			
	PEM $<5^{th}$ C	Undernourished $<2.3^{rd}$ C	Well-nourished $>2.3^{rd}$ $<85^{th}$ C	Overnourished $\geq 85^{th}$,95^{th} C	Obese $\geq 95^{th}$ C
Baseline	3	2	1		
3 months	6	1	4		1
6 months	4		4		
9 months	3		2	1	
12 months	3		3		
18 months	1		1		
24 months	3	1	1	1	

(i) Prevalence of malnutrition

PEM, established by using UAMA, was prevalent from the time of recruitment for a period of 2 years. Figure 4.16 shows that PEM was most prevalent at 3 months (10%) and 24 months (12.5%), whilst at 30 and 36 months no patients were classified as PEM. Table 4.6 describes the number of patients which were identified as PEM by UAMA, and those are compared against the classification established by BMI (undernourished, overnourished and obese). Figure 4.17 shows the prevalence of malnutrition identified by UAFA in all paediatric cancer patients.

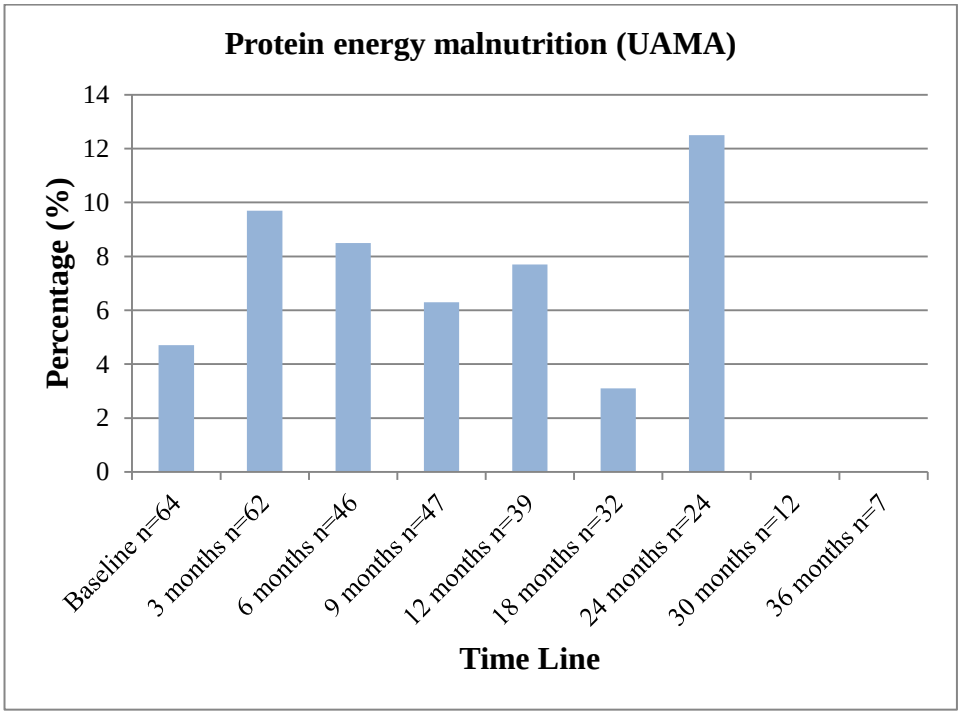

Figure 4.36 Prevalence (expressed as a percentage) of protein energy malnutrition in paediatric cancer patients at different stages of the disease

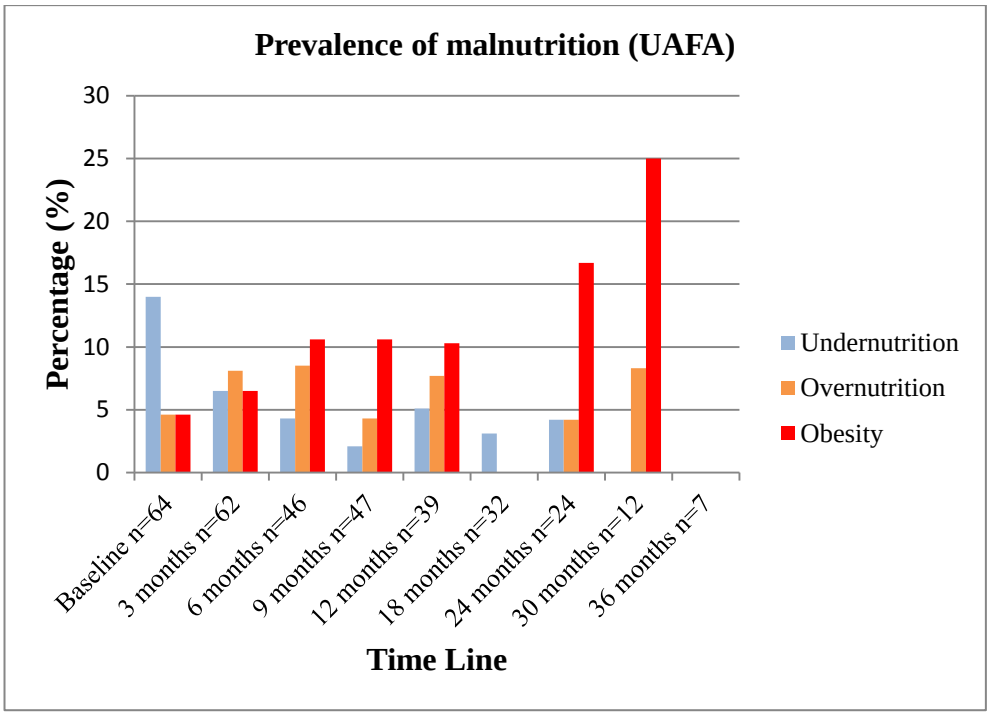

Figure 4.47 Prevalence of malnutrition established using UAFA in all paediatric cancer patients at different stages of the disease

The percentage of the 50th centile for UAMA and UAFA was stratified by gender and by diagnostic category. There were no statistically significant differences between males and females at any stage from baseline up to 36 months in neither the UAMA nor the UAFA data (U; $p>0.05$). UAMA and UAFA were compared between diagnostic categories using the Kruskal Wallis test at every stage from baseline up to 24 months only. No comparison was performed after that due to the small sample size. Children with solid tumours had significantly lower values of UAMA at 3 months in compare to the other 3 diagnostic categories [$H(3) = 8.844$, $p<0.03$]; however there were not statistically significant differences in the UAFA between groups at any stage from baseline up to 24 months. Jonckheere's test revealed a significant negative trend in both the UAMA and UAFA data at baseline [UAMA: J=840, z=-249, p=0.006, 95% CI 0.004-0.008; UAFA: J=766, z=-2.28, p<0.02, 95% CI 0.012-0.016] and 3 months [UAMA: J=792, z=-2.6, p=0.004, 95% CI 0.002-0.005; UAFA: J=701, z=-1.86, p<0.04, 95% CI 0.29-0.36] (figures 4.18, 4.19)

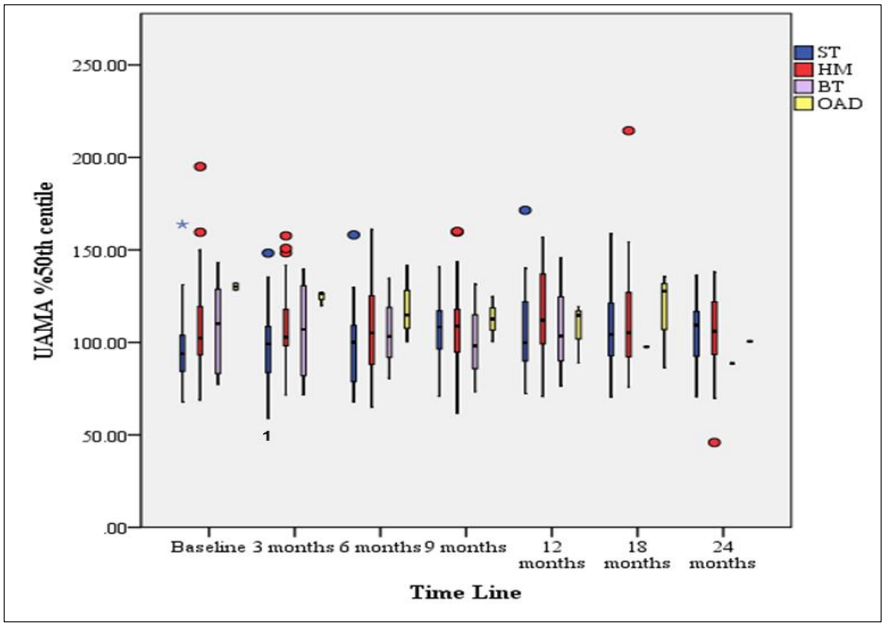

Figure 4.58 UAMA percentage of the 50th centile at different stages of the disease and stratified by diagnostic category

[1] Solid tumours had significantly lower UAMA than any other diagnosis (H (3); p<0.03) at 3 months. N values for the different diagnostic criteria and at different time points are presented in table 4.5, TSF values.

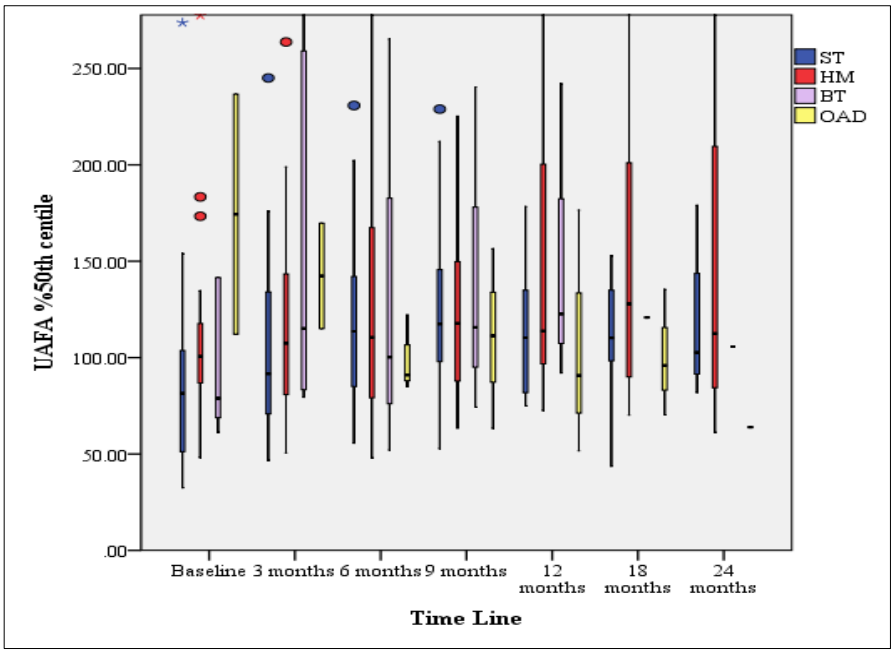

Figure 4.69 UAFA percentage of the 50th centile at different stages of the disease and stratified by diagnostic category

N values for the different diagnostic criteria and at different time points are presented in table 4.5, TSF values.

(ii) Changes in body composition at different stages of the disease

The percentage of FFM (FFM%) and FM (FM%) was calculated from both arm anthropometry (AA) measurements and BIA at every stage of the disease. Both measurements showed a gradual decrease in FFM% from baseline (FFM% AA: 69%; BIA: 68.5%) until the 12 month follow up (FFM% AA: 63%; BIA: 65%). This then remained steady for the remaining follow up period; 36 months (FFM% AA: 63; BIA: 64%). In contrast, the percentage of FM (FM%) increased gradually from baseline (FM% AA: 30%; BIA: 32%) until the end of the study period (FM% AA: 36%; BIA 37%) (Figures 4.20 and 4.21). No significant differences were found between diagnostic criteria and gender in either FFM% and FM% [gender: F(0.02), p=0.9; diagnoses: F(1.7), p=0.2] Figure 4.22 shows the agreement between FM% obtained from AA and BIA with a mean difference of 0.09% and 95% CI -1.0 to 1.2.

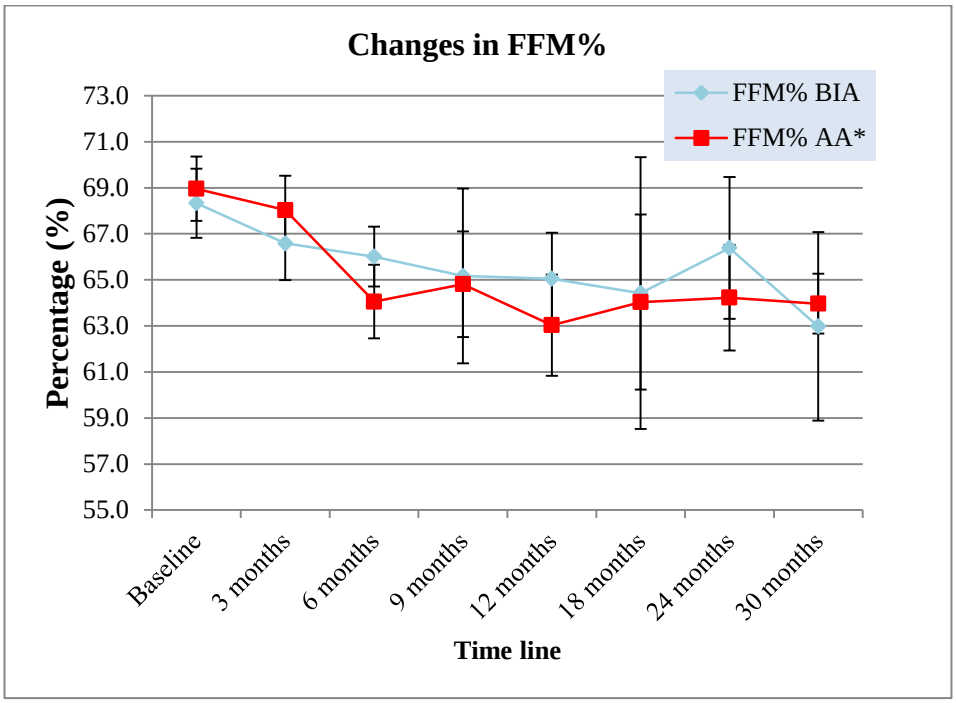

Figure 4.20 Changes in FFM% calculated from BIA and *arm anthropometry (AA**)**

N values for BIA and AA (see TSF) are presented in table 4.4

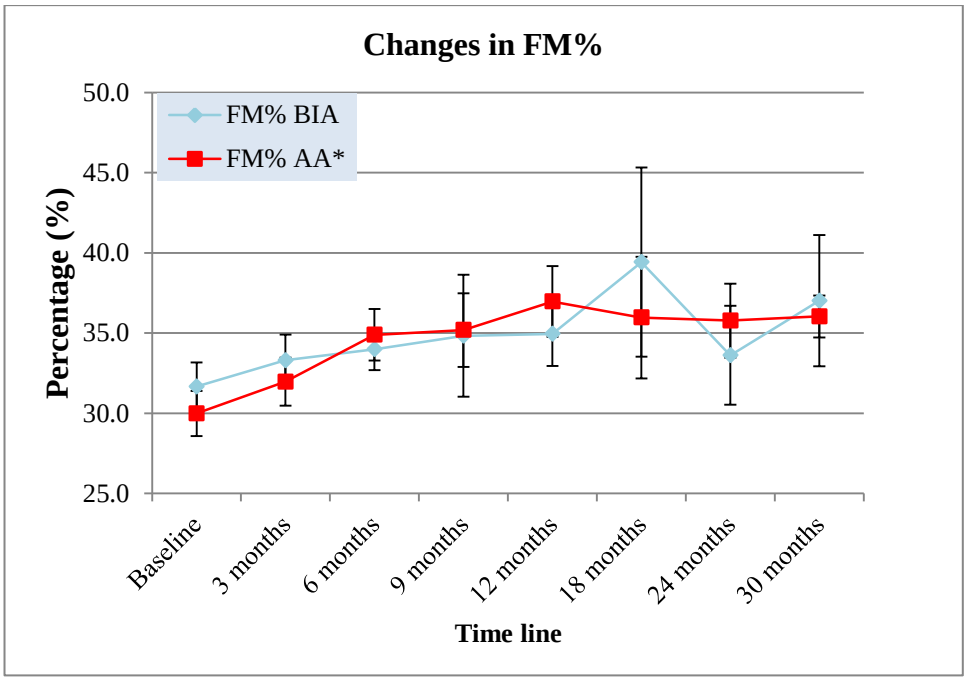

Figure 4.71 Changes in FM% calculated from BIA and *arm anthropometry (AA)

N values for BIA and AA (see TSF) are presented in table 4.4

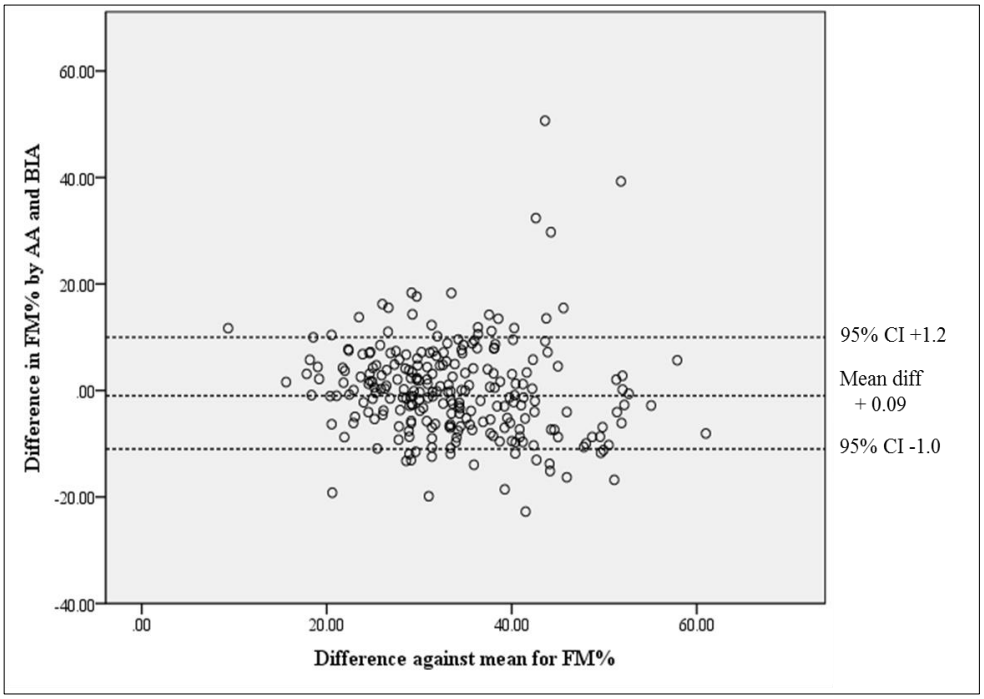

Figure 4.82 Agreement between FM% obtained from AA and BIA measurements

4.3.3 Linear growth

Linear growth was assessed using height for age (HFA) in centiles at every stage from the time of diagnosis up to 36 months. Data were presented including all cancers (i), stratified by gender (ii) and by diagnostic criteria (iii): (i) the median HFA centiles of paediatric cancer patients was above the 50th centile [median 55, IQR 26.5-740] at the time of diagnosis and this decreased at every stage until it reached a nadir after 6 months of treatment [median 41, IQR 23-64]. From this point HFA centiles increased steadily until it reached a peak at 30 months [HFA 69.5, IQR 47.7-93.7] (figure 4.23). (ii) A series of Mann-Whitney tests showed that there were no statistically significant differences between the HFA centiles of males and females at any stage (figure 4.24). However, the HFA centiles of females were consistently lower than that of the males at every stage after 3 months of treatment. (iii) Children diagnosed with solid tumours had the highest HFA at the time of diagnosis [median 62, IQR 25-76] and recruitment [median 62.5, IQR 35.5-79.7] (figure 2.25), whilst those diagnosed with haematological malignancies had the lowest HFA centiles at the time of recruitment [median 36, IQR 17.5-36]; however, it did not reach significance (Kruskal-Wallis test, $p>0.05$) (figure 4.24). This group remained with the lowest HFA centiles throughout the study period for 24 months; at which point it increased considerably reaching the 70th centile (IQR 32-86.9) and levelling with the other cancer groups (figure 4.26).

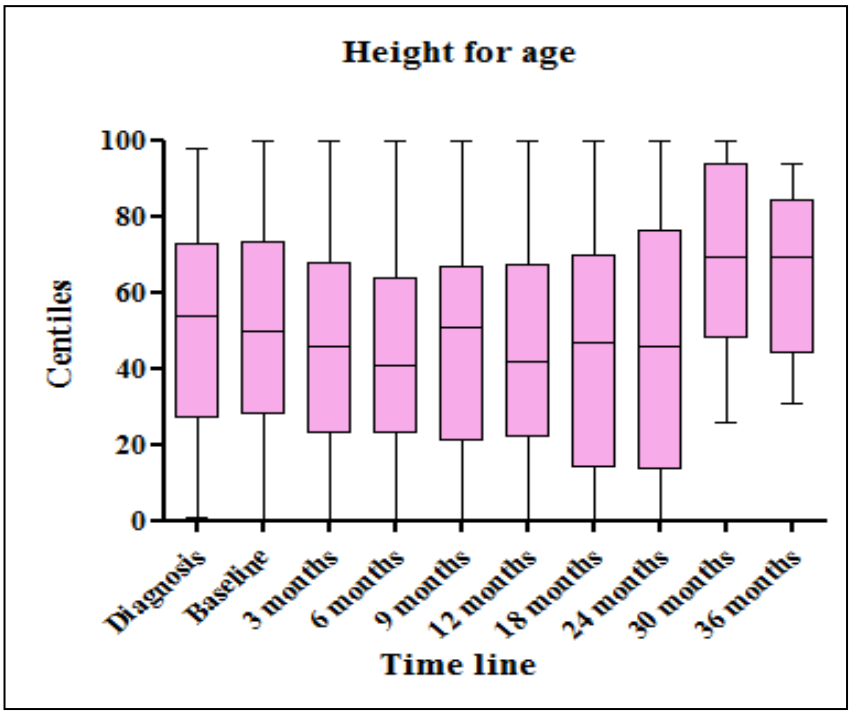

Figure 4.93 Height for age in paediatric cancer patients at different stages of disease. Data presented in median and 95% CI

For height for age n values at different time points see table 4.4.

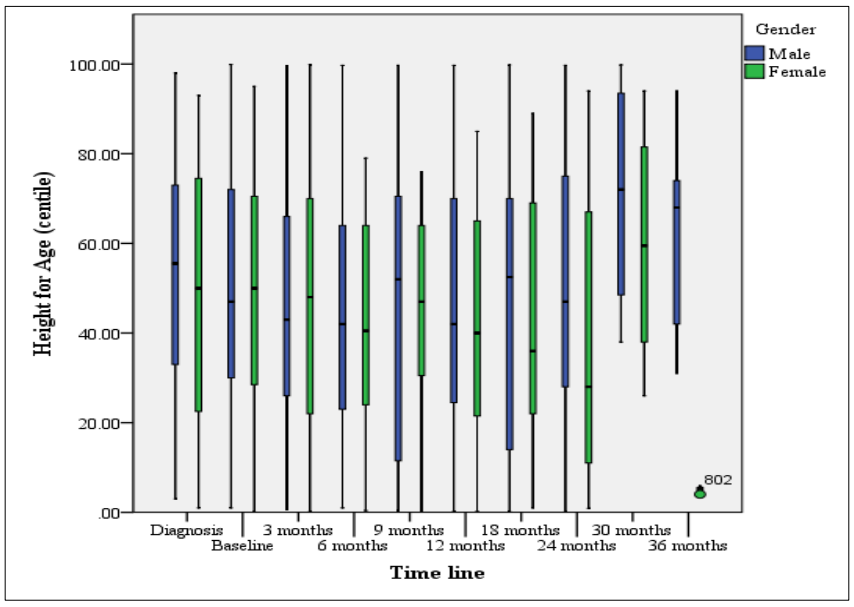

Figure 4.104 Height for age stratified by gender. Data presented in median and 95% CI

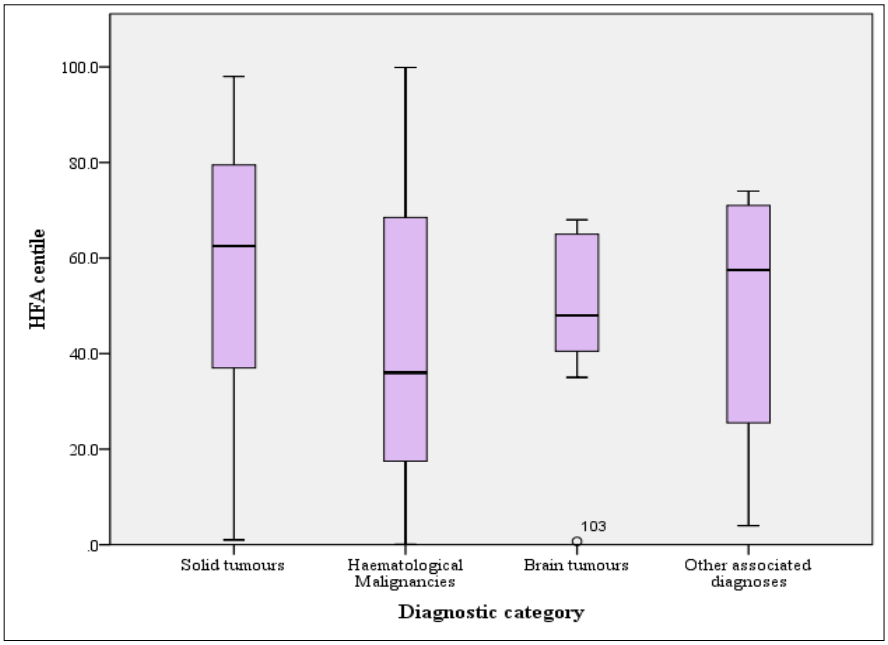

Figure 4.115 Height for age stratified by type of cancer at the time of recruitment

Solid tumours: n=36; Haematological malignancies: n=35; Brain tumours: n=7; OAD: n=4

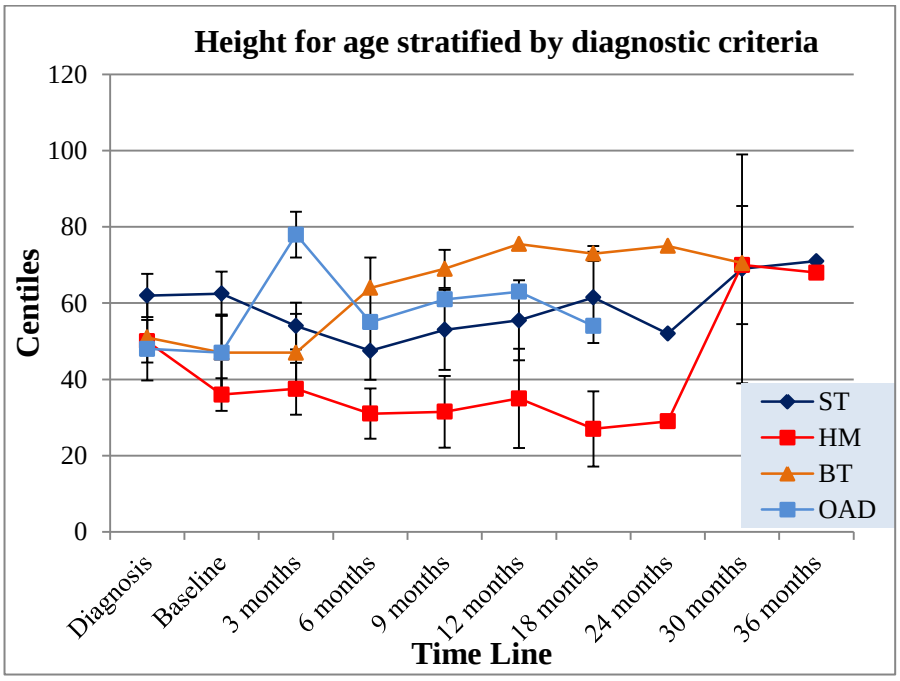

Figure 4.126 HFA centiles at different stages of the disease and data stratified by diagnostic criteria

All data are presented in median. ST: solid tumours; HM: Haematological malignancies; BT: Brain tumours; OAD: Other associated diagnoses (LCH). For HFA n values at different time points see table 4.4.

4.3.4 Patterns of change in nutritional status

Multilevel analyses (multilevel growth model) were performed to establish whether there were significant patters of change from baseline up to 18 months (table 4.7). No analysis was performed from 18 months onwards due to the reduced sample size from the later stages. Although the median BMI of the whole cohort decreased marginally at 3 months, multilevel analysis showed a significant increase in mean BMI of 1.5th centiles at 3 months and of 19th centiles at 18 months. BMI also increased significantly at 18 months (see table 4.7). For changes in FFM% and FM% see table 4.7.

Table 4-7 Mean changes in nutritional status established in 3, 9 and 18 months intervals.

	0-3 months			**0-9 months**			**0-18 months**		
Growth	Change	95% CI	*p* value	Change	95% CI	*p* value	Change	95% CI	*p* value
BMI C	1.5	1.31-3.47	<0.001	15.9	-0.1-0.2	0.6	19	1.31-3.47	<0.001
HFA C	-1.3	-5.9-0.04	0.05	-3.9	-0.4-0.02	0.07	-4.2	-0.005-0.24	0.06
Body composition									
FFM% AA	-0.9%	-1.6-(-0.5)	<0.001	-4.1%	-0.09-0.06	0.7	-4.87%	-0.01-0.02	0.7
FFM% BIA	-1.71%	-0.78-(-0.01)	<0.05	-2.83%	-0.13-(-0.01)	<0.02	-3.88%	-0.01-0.01	0.8
FM% AA	1.92%	-0.01-0.02	0.8	5.22%	-0.06-0.08	0.7	5.98%	-0.01-0.02	0.8
FM% BIA	1.71%	0.006-0.077	<0.05	3.1%	0.01-0.1	<0.02	3.9%	-0.01-0.01	0.4

All calculations were performed using multilevel analysis.

4.3.5 Factors related to changes in nutritional status

Multilevel analyses (mixed multilevel model) were performed (figure 4.27). Potential factors contributing to malnutrition, which was established by using BMI centile (the primary outcome), were: diagnostic criteria, treatment risk, age at diagnosis, nutritional support and TEI.

BMI centile 0-3 months	Fixed effects			
	Estimate	df	95% CI	P value
Intercept	48.4	79	41-55	<0.001
Time	2.4	44	1.31-3.47	<0.001
Diagnosis	8.1	75	-0.41-(-1.1)	0.04
Age	0.3	44	-1.3-1.8	0.7
Risk	-16.5	75	-24.3-(-8.6)	<0.001
TEI	0.02	51	0.002-0.2	0.02
NS	-6.4	74	-20.6-7.8	0.4

BMI centile 0-9 months	Fixed effects			
	Estimate	df	95% CI	P value
Intercept	44.13	317	32.3-55.9	<0.001
Time^3	0.04	296	-0.1-0.2	0.6
Diagnosis	1.2	48	-6.4-8.9	0.7
Age	0.85	48	-1.27-1.4	0.9
Risk	6.5	48	-1.91-14.9	0.13
NS	2.1	46	-10.7-14.9	0.7
TEI	0.003	46	-0.006-0.01	0.5

BMI centile 0-18 months	Fixed effects			
	Estimate	df	95% CI	P value
Intercept	72	284	34.7-109.5	<0.001
Time^5	-0.02	277	-0.07-0.02	<0.3
Diagnosis	1.15	7.7	-11.7-14.01	0.8
Age	0			
Risk	2.24	33	-18.8-23.25	0.7
NS	14.3	32	-2.3-31	0.09
TEI	0.004	33	-0.01-0.02	0.6

BMI centile 0-3 months	Conditional model			
	Estimate	df	95% CI	P value
Intercept	37.9	89.1	5.7-70.1	0.02
Time				
Diagnosis	-0.7	67.1	-8.9-7.5	0.8
Age				
Risk	-8.6	67.3	-16.8-(-0.4)	0.04
TEI	0.008	69.9	-0.001-0.002	0.09

Figure 4.137 Factors related to nutritional status changes at different stages of the disease

Fixed models (left) and conditional model (right).

4.3.6 Haematological and biochemical blood results

The haematological blood results haemoglobin, white cell count and platelets are presented as mean ± SD in table 4.8. These blood parameters cannot be interpreted for nutritional purposes due to the nature of the treatment of these patients, as they are treated with continuous cycles of chemotherapy (reduce these parameters) followed by blood transfusions (increase again).

Liver function was assessed by measuring alanine transaminase (ALT), alkaline phosphatase (ALP), G-glutamyl transferase (GGT) and bilirubin (table 4.9). ALT was elevated throughout the whole study period in the haematological and solid

tumour group in a large percentage of children, whilst those diagnosed with brain tumours and other associated diagnoses (OAD) had elevated levels only during the first 3 (OAD) and 6 months (BT) of treatment. Likewise GGT was elevated in a significant percentage of patients for the haematological malignancy, solid tumour and brain tumour group throughout the study period, whilst those diagnosed with OAD had elevated levels only at 6 months. As it can be seen in table 4.9 ALP levels were reduced in many patients throughout the study period, especially in children diagnosed with haematological malignancies, solid tumours and OAD. 80% (4/5) of children diagnosed with brain tumours had elevated ALP levels at diagnosis and all had normal levels thereafter. Finally bilirubin was only elevated in the haematological malignancy group from baseline up to 30 months.

Kidney function was assessed by measuring plasma creatinine and urea (table 4.10). Both parameters were variable (elevated and reduced levels) throughout the study period in all diagnostic categories.

Inflammatory markers were tested by measuring standard sensitivity C-Reactive protein (ssCRP), albumin (acute phase reactant) and ferritin (table 4.11). ssCRP was highest in the solid tumours throughout the study period, whilst both CRP and ferritin tended to be high in children diagnosed with haematological malignancies also throughout the study period. In contrast, plasma albumin tended to be below the reference range in the haematological malignancy, solid tumour and OAD diagnoses group during the first year of treatment only, whilst brain tumours had normal levels throughout the study period.

Table 4-8 Haematological blood results presented as mean ± standard deviation (SD)

Blood analysis	Time months	Haematological malignancies		Solid tumours		Brain tumours		Other malignancies	
Haemoglobin g/L		**n**	**Mean ± SD**	**n**	**Mean ± SD**	**n**	**Mean ± SD**	**n**	**Mean ± SD**
Birth 149-237	0	34	99.4±15.0	34	104.6±14.3	5	105.4±12.5	4	88.2±23.6
2 weeks 134-198	3	31	102.5±14.5	29	104.9±15.6	4	93.8±6.8	3	96.0±7.0
6 months 111-141	6	19	105.6±12.1	16	102.1±11.3	2	94.0±7.1	4	98.0±6.2
1 year 113-141	9	15	108.9±20.7	9	118.2±16.9	3	102.3±14.6	4	101.5±7.3
2-6 years 115-155	12	13	109.1±13.3	6	106.3±18.2	3	96.0±4.6	2	105.0±17.0
6-12 years 115-155	18	15	109.9±17.1	4	117.8±16.7	1	97.0	1	103.00
12-18 years	24	10	117.3±9.6	1	125	1	79.0	1	104.00
Males: 130-160	30	5	119.0±11.7	1	116	1	96.0	0	-
Females: 120-160	36	3	118.3±8.7	0	-	0	105.4	0	-
White Cell Count	0	34	2.7±2.6	34	5.6±4.2	5	2.6± 5.7	4	4.2±2.0
5-17 x 109 /L	3	31	4.5±4.0	28	6.9±14.9	4	4.0±5.2	2	4.5±0.1
	6	19	3.6±2.4	16	3.6±2.2	2	2.4±4.9	4	3.9±4.5
	9	15	2.7±1.4	10	4.2±2.3	3	1.4±6.3	4	5.4±3.2
	12	13	3.1±1.9	6	5.3±3.4	3	2.0±5.9	2	7.4±2.3
	18	15	3.6±2.4	4	4.7±3.5	1	5.5	1	0.5
	24	10	2.5±0.7	1	2.6	1	0.7	1	16.3
	30	5	3.5±2.5	1	2.9	1	2.2	0	-
	36	3	2.6±1.7	0	5.6	0	1.7	0	-
Platelets	0	34	148.9±122.4	34	279.6±175.7	5	389.80±125.1	4	266.5±176.8
150-400 x 10^9/L	3	31	214.4±134.8	29	224.9±118.1	4	278.25±281.8	3	347.7±291.6
	6	19	201.5±146.0	16	223.9±147.3	2	366.00±247.5	4	302.2±210.3
	9	15	188.5±97.4	10	217.0±126.3	3	360.67±144.3	4	319.2±129.6
	12	13	222.8±96.7	6	207.5±158.1	3	366.67±105.6	2	306.0±209.3
	18	15	191.4±87.6	4	127.2±87.1	1	345.00	1	175.0±176.7
	24	10	185.2±84.1	1	156.0	1	333.00	1	427.0
	30	5	194.8±102.7	1	315.0	1	473.00	0	266.5
	36	3	198.7±83.2	0	-	0	389.80	0	347.7

Table 4-9 Blood parameters showing liver function. Values are expressed as mean ± SD and stratified by diagnostic criteria

Blood analyses	Time months	Haematological malignancies			Solid tumours			Brain tumours			Other associated diagnoses		
Liver function test		**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**
Alanine	0	34	86.9±95.7	↑14(41)	32	28.0±16.2	↑2(6)	5	85.0±68.6	↑3(60)	4	18.0±5.6	↑1(25)
transaminase	3	32	85.7±99.3	↑16(50)	31	35.3±27.1	↑6(19)	4	41.2±25.4	↑1(25)	3	32.3±31.1	↑1(33)
(ALT) unit/L	6	19	85.0±59.1	↑11(58)	17	42.1±30.2	↑5(29)	2	58.0±67.9	↑2(100)	4	31.2±25.6	0
0-12m <65.0	9	13	107±90.8	↑9(69)	9	58.6±67.0	↑3(33)	3	28.3±14.7	0	4	19.0±4.8	0
1-16y <50.0	12	14	57.8±32.8	↑8(57)	6	27.5±17.0	↑1(17)	3	28.7±11.8	0	2	18.0±4.2	0
	18	14	56.1±30.0	↑8(57)	4	42.2±26.2	↑2(50)	1	12.0	0	1	37.0	0
	24	10	108±121.0	↑8(80)	1	110.0	↑1(100)	1	18.0	0	1	18.0	0
	30	5	90.2±78.3	↑3(60)	1	374.0	↑1(100)	1	20.0		0	-	
	36	3	27.7±6.6	0	0	-	0	0	-		0	-	
Alkaline	0	34	163±99	↑1(3) ↓6(18)	34	127±49.5	↓6(18)	5	219±98	↑4(80)	4	158±84	↓1(25)
Phosphatase	3	32	179±104	↑1(3) ↓3(9)	31	150±57.7	↓1(32)	4	158±32	0	3	274±202	↑1(33)
(ALP) unit/L	6	19	133±43	↓3(16)	17	171±51.6		2	192±61	0	4	164±81	↓1(25)
0-1m 80-440	9	14	176±102	0	10	387±699.0	↑1(10)	3	165±44	0	4	187±46	0
1-6m 120-580	12	14	156±48	↓1(7)	6	216±105.2	↓1(17)	3	142±37	0	2	163±0.7	0
6m-1y 110-430	18	15	723±2188	↑1(7)	4	222±46.0		1	119	0	1	142	0
1-12y 100-400	24	10	184±111	↑1(10) ↓1(10)	1	88.0	↓1(100)	1	109	0	1	155	0
12-16y	30	5	132±59	0	1	221	0	1	112	0	0	-	
M 100-400	36	3	142±18	0	0	-	0	0			0	-	
F 60-400													
G-glutamyl	0	34	68.6±103	↑9(26)	32	47.5±116	↑5(16)	5	38.8±34.9	↑2(40)	4	24.2±14.4	0
transferase	3	32	43.3±58	↑6(17)	31	26.8±18	↑5(16)	4	34.5±23.1	↑2(50)	3	24.0±24.3	0
(GGT) unit/L	6	19	45.0±62	↑6(32)	17	56.1±77	↑5(29)	2	51.0±4.2	↑2(100)	4	19.7±15.7	↑1(25)
0-2m <200	9	13	86.1±215	↑4(31)	10	75.9±198	↑1(10)	3	25.3±22.3	↑1(33)	4	13.7±9.1	0
1-16y <40.0	12	14	40.8±61	↑3(21)	6	38.5±64	↑1(33)	3	26.3±13.6	0	2	15.5±10.7	0
	18	14	27.0±23	↑4(27)	4	67.0±83	↑2(50)	1	14.0	0	1	30.0	0

	24	10	21.1±14	↑1(10)	1	33.0	0	1	14.0	0	1	23.0	0
	30	5	30.0±27	↑1(20)	0	-	0	1	9.00	0	0	-	-
	36	3	16.7±11.8	0	0	-	0	0	-		0	-	-
Bilirubin	0	34	13.9±6.8	↑7(21)	32	7.5±5.9	0	5	4.8±1.3	0	4	6.2±4.8	0
µmol/L	3	32	10.8±8.4	↑5(16)	31	7.9±4.6	0	4	9.2±3.1	0	3	4.3±2.3	0
All ages <20.0	6	19	11.0±9.1	↑2(10)	17	6.8±3.3	0	2	8.0±2.8	0	4	4.7±2.1	0
	9	14	17.5±19.5	↑4(29)	10	5.4±2.8	0	3	8.0±1.7	0	4	5.5±3.1	0
	12	14	16.4±15.1	↑4(29)	6	5.3±2.2	0	3	6.7±2.1	0	2	5.5±3.5	0
	18	15	15.8±13.9	↑3(20)	4	8.2±4.0	0	1	6.0	0	1	5.0	0
	24	10	14.7±7.7	↑3(30)	1	24.0	↑1(100)	1	4.0	0	1	3.0	0
	30	5	28.4±21.8	↑3(60)	1	10.0	0	1	4.0	0	0	-	-
	36	3	18.3±11.3	0	0	-	-	0	-	-	0	-	-

M: months; y: years; ↑high levels; ↓ low levels; Reference ranges: laboratory handbook (NHS Scotland Laboratory Handbook)

Table 4-10 Blood parameters showing kidney function. Values are expressed as mean ± SD and stratified by diagnostic criteria

Blood analyses	Time months	Haematological malignancies			Solid tumours			Brain tumours			Other associated diagnoses		
Kidney function test		**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**
Urea mmol/L	0	34	5.7±2.6	↑10(29)	34	5.0±8.0	↓7(21) ↑2(6)	5	3.4±1.1	↑1(20)	4	4.1±0.8	↓1(25)
14d-1m 0.08-0.39	3	32	4.0±1.6	↓1(3) ↑2(6)	31	3.5±1.5	↓4(13)	4	3.2±2.0	↓1(25)	3	3.2±1.2	0
1m-12m 1.2-5.0	6	19	3.5±1.4	↓3(16)	17	4.2±2.4	↓2(12) ↑2(12)	2	4.0±1.3	0	4	4.0±1.6	0
1-16y 2.4-6.5	9	14	3.4±1.6	↓2(14)	10	5.4±3.7	↑1(10)	3	3.8±1.0	0	4	4.0±0.7	0
	12	14	3.5±0.9	↓1(7) ↑1(7)	6	5.5±4.8	↓1(17)	3	3.2±1.1	0	2	4.6±2.3	0
	18	15	3.4±1.5	↓3(20)	4	6.4±5.8	↑1(25)	1	2.2	0	1	2.5	0
	24	10	4.7±3.1	↓1(10) ↑1(10)	1	1.9	↓1(100)	1	2.6	0	1	3.8	0
	30	5	4.4±0.5	0	1	4.1	0	1	5.5	0	0	-	0
	36	3	3.6±0.5	0	0	5.0	0	0	-	0	0	-	0
Creatinine µmol/L	0	34	26.2±12.8	↓4(12) ↑1(3)	34	27.2±11.5	↓3(9)	5	26.2±10.3	0	4	27.2±12.9	0
0-7d not defined	3	32	28.0±12.5	↓1(3) ↑1(3)	31	35.3±17.5	↑2(6)	4	29.7±13.3	0	3	18.7±7.1	↓1(33)
7-28d 15-39	6	19	28.2±12.8	↓1(5) ↑1(5)	17	33.5±14.9	↑1(6)	2	39.5±6.5	0	4	25.5±10.1	0
1-12m 12-31	9	14	28.4±9.6	↓2(14)	10	43.6±30.4	↑1(10)	3	46.0±13.5	↑1(33)	4	27.0±11.8	0
1y 13-28	12	14	30.9±9.0	↓1(7)	6	51.3±64.0	↑1(17)	3	35.7±3.8	0	2	36.5±20.5	↑1(50)
2y 15-34 3y 15-42	18	15	30.7±12.1	↓3(20) ↑1(7)	4	47.5±43.8	↑1(25)	1	38.0	0	1	14.0	0
4-6y 15-42 7-11y 26-57 12-14y 31-67	24	10	32.1±13.3	↓1(10) ↑1(10)	1	40.0	0	1	32.0	0	1	25.0	0
15-18y	30	5	32.0±2.7	0	1	25.0	↓1(100)	1	34.0	0	0	-	-
M 39-92 F 34-72	36	3	24.0±17.6	0	0	-	0	0	-	0	0	-	-

Table 4-11 Acute phase reactant parameters. Values are expressed as mean ± SD and stratified by diagnosis criteria

Blood analyses	Time months	Haematological malignancies			Solid tumours			Brain tumours			Other associated diagnoses		
Acute phase reactants		**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**
Ferritin	0	30	708±563	↓1(3) ↑28(93)	32	526±510	↓1(3) ↑28(87)	7	288±270	↑3(43)	3	45.7±53	↓1(33) ↑19(33)
µg/L	3	30	1750±1596	↑29(97)	30	1208±2199	↑24(80)	4	438±407	↑3(75)	1	2364	↑1(100)
0-5y 12-80	6	19	1981±2524	↑17(89)	16	1688±1576	↑14(87)	2	845±1020	↑2(100)	4	294±448	↓2(50) ↑1(25)
5-16y 15-80	9	13	4841±11029	↑13(100)	10	1299±2284	↑8(80)	3	325±355	↑2(67)	4	328±614	↓1(25) ↑1(25)
	12	12	3590±5636	↓1(8) ↑11(92)	6	1828±2962	↑6(100)	3	332±176	↑3(100)	2	19±18	↓1(50)
	18	14	2183±2127	↑13(93)	4	2128±2235	↑4(100)	1	195	↑1(100)	1	885	↑1(100)
	24	10	2534±4052	↑10(100)	1	2273	↑1(100)	1	296	↑1(100)	1	52	0
	30	5	1290±1385	↑5(100)	0	-	-	1	126	↑1(100)	0	-	-
	36	3	1404±2098	↑2(67)	0	-	-	0	-	-	0	-	-
Albumin	0	34	28.5±5.1	↓16(47)	33	32.4±6.3	↓7(21)	5	36.2±2.7	0	4	32.5±9.3	↓1(25)
g/L	3	32	36.1±4.5	↑1(31)	29	34.8±5.8	↓1(3)	4	37.0±3.6	0	3	34.0±6.9	↓1(33)
0-3m maturity	6	19	36.8±5.9	↓1(5)	16	35.3±4.8	↓1(6)	2	40.0±1.4	0	4	33.0±7.3	↓1(25)
dependent	9	15	36.4±5.1	↓1(7)	10	36.5±4.8	↓1(10)	3	39.7±2.5	0	4	37.0±3.1	0
3-12m 27-42	12	14	36.7±5.9	↓1(7)	6	33.7±3.9	0	3	39.3±2.3	0	2	40.5±0.7	0
1-16y28-45	18	15	36.9±3.7	0	4	33.7±7.4	↓1(25)	1	41.0	0	1	31.0	0
	24	10	36.5±2.0	0	1	37.0	0	1	37.0	0	1	37.0	0
	30	5	37.0±1.6	0	1	41.0	0	1	38.0	0	0	-	0
	36	3	38.3±2.9	0	0	-	-	0	-	-	0	-	-
[1]ssCRP	0	30	12.2±25.0	↑12(40)	30	28.3±52.9	↑13(43)	5	1.3±0.4	↑2(40)	3	4.7±6.3	↑1(33)
mg/L	3	28	11.1±27.3	↑6(21)	30	8.0±14.4	↑10(33)	4	41.0±45.1	0	2	7.5±9.2	↑1(50)
All ages <5	6	18	14.6±49.3	↑3(17)	15	15.4±27.8	↑6(40)	2	2.5±2.1	↑1(50)	4	6.8±8.3	↑1(25)
	9	13	5.1±9.1	↑2(15)	10	4.9±6.0	↑2(20)	3	2.7±2.9	↑1(33)	4	1.2±0.5	0
	12	11	19.1±53.4	↑2(18)	6	4.8±3.2	↑3(50)	3	6.0±5.0	↑2(67)	2	3.5±2.1	0
	18	14	2.5±4.5	↑1(7)	4	4.5±3.5	↑2(50)	1	1.0	0	1	2.0	0
	24	10	2.6±4.4	↑1(10)	1	1.0	0	1	1.0	0	1	1.0	0
	30	5	8.0±12.9	↑1(20)	0	-	-	1	1.0	0	0	-	-

	36	3	8.7±13.3	↑1(33)	0	-	-	0	-	-	0	-	-

[1]ssCRP: standard sensitivity C-Reactive Protein; Reference ranges: laboratory handbook (NHS Scotland Laboratory Handbook).

4.3.7 Full blood nutritional screening

4.3.7.1 Antioxidant micronutrients status

Both plasma vitamins A and E/Ch were assessed (table 4.12). Abnormal vitamin A levels, both elevated and reduced, were prevalent in all diagnostic groups and throughout the study period. There were significant but weak correlations between plasma vitamin A (mmol/L) and the following variables: GGT ($r=0.1$; $p=0.04$), albumin ($r=0.2$; $p<0.001$), BMI centiles ($r=0.2$; $p<0.001$) and total vitamin A intake (µg/day) ($r=0.15$; $p=0.02$). Spearman's test showed a negative and moderate significant correlation between plasma vitamin A and ssCRP ($r=0.3$; $p<0.001$). However, no statistically significant correlation was obtained between vitamin A levels and ALT ($r=0.03$; $p=0.7$). In order to establish whether supplementation was associated with hypo and hypervitaminosis A, the data was stratified by groups (micronutrient supplementation only, macronutrient supplementation only and a combination of both). No statistical significant associations were established between vitamin A supplementation and neither hypervitaminosis A [Fisher Exact test (3.6); $p=0.3$] nor hypovitaminosis A status [Fisher Exact test (2.8); $p=0.4$].

Plasma vitamin E/Ch was abnormal only in very few patients and results from plasma copper, selenium and zinc are presented in table 5.10. Plasma vitamin E/Ch correlated significantly with vitamin E intakes [$r=0.2$ (weak, positive); $p=0.007$].

4.3.7.2 Non-antioxidant micronutrients status

Plasma folate and vitamin B12 were assessed. As it can be seen from table 4.13 serum folate was reduced, especially in the haematological malignancy group, throughout the study period. Finally, results from plasma vitamin B12 were variable as patients with haematological malignancies, solid tumours and OAD had both hypovitaminosis and hypervitaminosis B12.

Table 4-12 Blood results: antioxidant micronutrient status at different stages of disease and treatment. Values expressed as mean±SD

Blood analyses	Time months	Haematological malignancies			Solid tumours			Brain tumours			Other associated diagnoses		
Fat soluble vitamins		**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**
Vitamin A	0	27	1.3±0.7	↓2(7) ↑5(18)	30	1.1±0.6	↓5(17) ↑3(10)	5	1.3±0.4	↑1(20)	2	1.2±0.0	0
µmol/L	3	28	1.5±0.8	↓2(7) ↑9(32)	28	1.4±0.6	↓3(11) ↑6(21)	4	0.9±0.4	↓1(25)	1	2.0	↑1(100)
0-12m 0.5-1.5	6	17	1.4±0.7	↓2(12) ↑4(23)	16	1.3±0.5	↓3(19) ↑3(19)	2	1.9±0.8	↑1(50)	4	1.4±0.9	↓1(25)
1-7y 0.7-1.5	9	14	1.7±0.8	↑6(43)	9	1.6±0.6	↑4(44)	3	1.3±0.1	0	4	1.5±0.7	↑1(25)
7-13y 0.9-1.7	12	13	1.7±0.9	↓1(8) ↑5(38)	6	1.5±0.8	↑2(33)	3	1.3±0.2	0	2	1.6±0.1	0
13-19y 0.9-2.5	18	14	1.6±0.8	↓2(14) ↑7(50)	4	1.9±0.6	↑3(75)	1	1.6	0	1	0.9	0
Toxic levels	24	9	1.6±0.5	↑4(29)	1	1.8	↑1(100)	1	1.3	0	1	1.9	0
>3.3	30	5	1.6±0.8	↑2(40)	0	-	-	1	1.7	0	0	0	-
	36	3	3.1±3.2	↑1(33)	0	-	-	0	-	-	0	0	-
Vitamin E/Ch	0	26	6.0±1.9	↓1(4) ↑1(4)	30	6.2±1.5	↑1(3)	5	5.3±0.6	0	2	7.6±4.2	↑1(50)
µmol/L	3	26	6.1±1.5	0	26	6.2±1.5	↑1(4)	4	5.1±1.0	0	1	6.1	0
All ages 0.25-2.00	6	17	5.5±1.0	0	16	6.3±1.3	0	2	6.1±0.0	0	4	5.4±0.7	0
	9	14	5.6±1.4	0	9	6.7±1.8	0	3	6.3±0.7	0	4	6.0±1.2	0
	12	13	5.3±1.3	↓1(8)	6	6.3±1.5	0	3	5.2±0.7	0	2	5.8±0.3	0
	18	14	5.3±1.0	0	4	6.0±2.4	0	1	5.6	0	1	3.1	↓1(100)
	24	9	5.1±1.3	↓1(11)	1	4.7	0	1	7.0	0	1	6.4	0
	30	5	5.7±1.5	0	0		0	1	6.6	0	0		0
	36	3	5.4±1.5	0	0		0	0	-	-	0		0
Minerals													
Copper	0	26	12.7±5.2	↓15(58)	34	20.5±6.6	↓2(6)	5	15.2±1.6	0	2	15.7±4.0	↑1(50)

				↑1(4)			↑14(41)						
µmol/L	3	26	17.1±4.0	↓5(2)	28	17.4±3.7	↑5(18)	4	21.2±5.2	↑1(25)	2	18.2±2.7	↑1(50)
				↑4(15)									
	6	19	16.1±4.7	↓6(32)	14	21.0±5.9	↑2(14)	2	18.3±2.3	0	4	17.0±6.4	↓1(25)
				↑2(10)									↑1(25)
	9	14	17.6±4.4	↓2(14)	10	18.6±3.5	0	3	17.1±2.2	0	4	16.9±3.6	0
				↑3(21)									
	12	13	15.6±4.0	↓2(15)	6	19.6±2.9	0	3	18.9±4.2	0	2	21.6±4.2	0
				↑2(15)									
	18	14	14.3±3.5	↓3(21)	3	15.6±1.1	0	1	14.0	0	1	15.7	0
				↑1(7)									
	24	9	15.8±3.7	↓2(22)	1	13.7	0	1	16.7	0	1	17.3	0
	30	5	15.1±6.9	↓3(60)	0			1	14.9	0	0		
	36	3	15.9±4.7	0	0			0			0		
Selenium	0	26	1.2±0.5	↓1(4)	34	0.9±0.3	↓2(59)	4	0.7±0.2	0	2	1.0±0.2	0
				↑8(31)			↑1(3)						
µmol/L	3	26	0.9±0.2	↓2(8)	28	0.9±0.2	↓3(11)	4	0.8±0.2	↓1(25)	2	0.8±0.3	0
				↑3(11)									
0-2y 0.2-0.9	6	19	0.9±0.2	↓1(5)	14	0.9±0.3	↓1(7)	2	0.7±0.0	0	4	0.9±0.2	0
							↑2(14)						
2-4y 0.5-1.3	9	14	0.9±0.2	↓2(14)	10	1.0±0.4	↓1(10)	3	0.8±0.1	↓1(33)	4	0.8±0.2	0
							↑2(20)						
4-16y 0.7-1.7	12	14	0.9±0.2	↓9(64)	6	1.0±0.3	↓4(67)	3	0.8±0.2	↓1(33)	2	0.9±0.4	↓2(100)
	18	14	1.0±0.1	0	3	0.9±0.3	0	1	0.6	↓1(100)	1	1.2	0
	24	9	1.1±0.2	0	1	0.8	0	1	0.7	0	1	1.0	0
	30	5	1.1±0.4	0	0			0			0		
	36	3	1.1±0.4	0	0			0			0		
Zinc	0	24	14.3±8.8	↓13(54)	32	11.0±3.6	↓23(72)	5	10.8±2.0	↓4(80)	2	8.3±0.7	↓2(100)
				↑4(17)			↑2(6)						
µmol/L	3	24	10.4±2.1	↓18(75)	26	11.6±2.5	↓15(58)	4	10.7±1.9	↓2(50)	2	11.4±0.2	↓2(100)
<16y 12-18	6	19	13.6±14.6	↓14(74)	11	12.1±5.5	↓8(73)	2	10.5±1.8	↓2(100)	4	10.6±1.3	↓4(100)
				↑1(5)			↑1(9)						
	9	13	11.8±5.1	↓10(77)	9	10.6±0.7	↓3(33)	3	11.7±1.4	↓2(67)	4	10.6±0.7	↓4(100)
				↑1(8)			↑1(11)						
	12	12	10.2±2.8	↓9(75)	6	10.8±3.4	↓4(67)	3	20.6±15.5	↓2(67)	2	10.6±0.1	↓2(100)

									↑1(33)			
18	14	10.6±1.9	↓12(86)	3	11.5±3.6	↓2(67)	1	11.3	↓1(100)	1	8.8	↓1(100)
24	8	10.3±2.5	↓5(62)	1	12.8	0	1	11.9	↓1(100)	1	12.3	0
30	4	11.1±1.9	↓3(75)	0		-	1	11.0	↓1(100)	0	-	-
36	3	13.5±1.2	↓0	0		-	0			0	-	-

Reference ranges: laboratory handbook (NHS Scotland Laboratory Handbook)

Table 4-13 Non-antioxidant micronutrient status. Values are expressed as mean ± SD and stratified by diagnostic criteria

Blood analyses	Time months	Haematological malignancies			Solid tumours			Brain tumours			Other associated diagnoses		
Water soluble vitamins		**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**	**n**	**Mean ± SD**	**Abnormal n(%)**
Vitamin B 12	0	25	729±592	↓2(8) ↑5(20)	29	488±205	↓1(3)	5	498±184	0	3	448±326	0
ng/L	3	28	624±361	↓1(4) ↑2(7)	29	740±501	↓3(10) ↑5(17)	4	558±177	0	2	753±154	0
	6	18	522±270	↓1(5) ↑1(5)	15	827±581	↑3(20)	2	530±257	0	4	456±310	↓1(25)
0-5y 280-1400	9	15	545±315	0	8	752±411	↓1(12) ↑1(12)	3	737±398	0	4	706±452	0
5-16y 200-1100	12	11	660±331	↑1(9)	6	627±317	↓1(17)	3	763±354	0	2	364±9.2	0
	18	13	524±343	↓1(8) ↑1(8)	4	836±215	0	1	671	0	1	764	0
	24	10	474±223	↓2(20)	1	196	↓1(100)	1	424	0	1	527	0
	30	5	553±136	0	0	-	-	1	652	0	0		-
	36	3	645±160	0	0	-	-	0		-	0		-
Serum folate	0	24	7.1±6.2	↓3(12) ↑1(4)	25	8.7±5.3	↓3(12)	5	9.1±2.4	0	3	6.2±4.6	↓1(33)
µg/L	3	28	8.3±5.8	↓5(18)	28	9.9±4.5	↓1(4)	4	13.1±6.8	0	2	9.0±4.7	0
1-12m >7	6	18	7.1±5.1	↓2(11)	16	9.3±4.1	↓1(6)	2	4.5±1.8	0	4	9.5±4.9	0
1-4y 4-30	9	15	6.8±3.5	↓1(7)	8	9.2±5.4	↓1(12)	3	8.6±6.7	↓1(33)	4	8.3±4.0	0
4-16y 3-20	12	11	6.0±3.0	↓1(9)	6	9.2±4.8	0	3	7.7±5.7	↓1(33)	2	10.9±2.1	0
	18	13	5.1±2.6	↓3(23)	4	7.2±4.2	0	1	4.5	0	1	13.5	0
	24	10	6.1±5.4	↓4(40)	1	2.4	↓1(100)	1	3.5	0	1	4.9	0
	30	5	7.0±3.2	0	0		-	1	9.2	0	0		-
	36	3	7.6±6.1	↓1(33)	0		-	0		-	0		-

Reference ranges: laboratory handbook (NHS Scotland Laboratory Handbook)

4.3.8 Nutrient intake and nutritional support

4.3.8.1 Energy and macronutrient intake

TEI were established at every stage from the time of recruitment up to 36 months and these were compared to the total energy requirements (TER). TER were estimated using the Henry equation (2005) and all data were adjusted for age, gender and physical activity (10^{th} centile). TEI of all paediatric cancer patients were consistently higher than TER at every stage of the disease apart from the 3 months follow up. Statistical significance was obtained from 9 months onwards (see figure 4.28). Protein, fat and carbohydrate intake was consistently higher than recommendations at every stage of the disease apart from the 3 months follow up (see figures 4.29, 4.30 and 4.31).

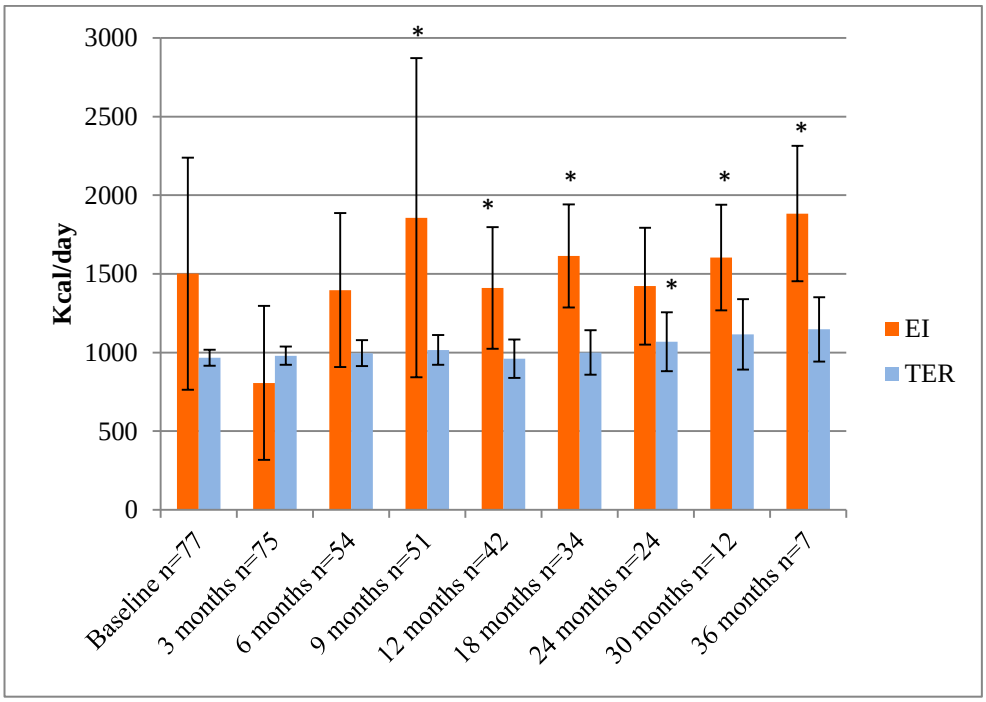

Figure 4.148 Energy Intake (EI) compared to total energy requirements (TER) in paediatric cancer patients (all diagnosis) at different stages of the disease

Values are expressed as mean ± SD; TER was calculated using Henry's equation (2005); *Indicates $p<0.05$ (Independent t test).

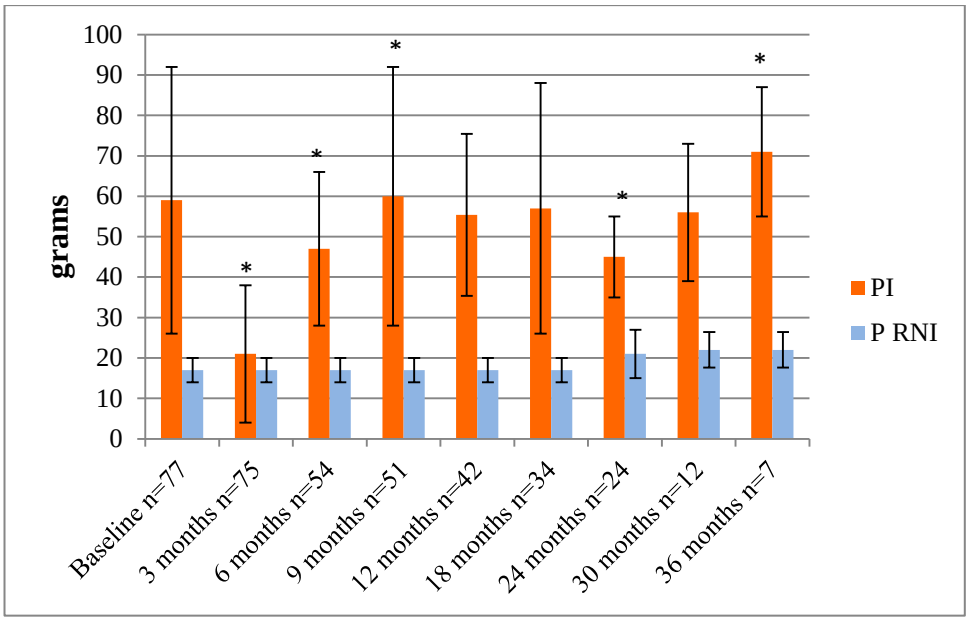

Figure 4.159 Protein intake (PI) compared to protein requirements (PR) in paediatric cancer patients (all diagnosis) at different stages of the disease

Values are expressed as mean ± SD; protein requirements obtained from RNI (Department of Health 1991); *Indicate p<0.05 (Independent t-test).

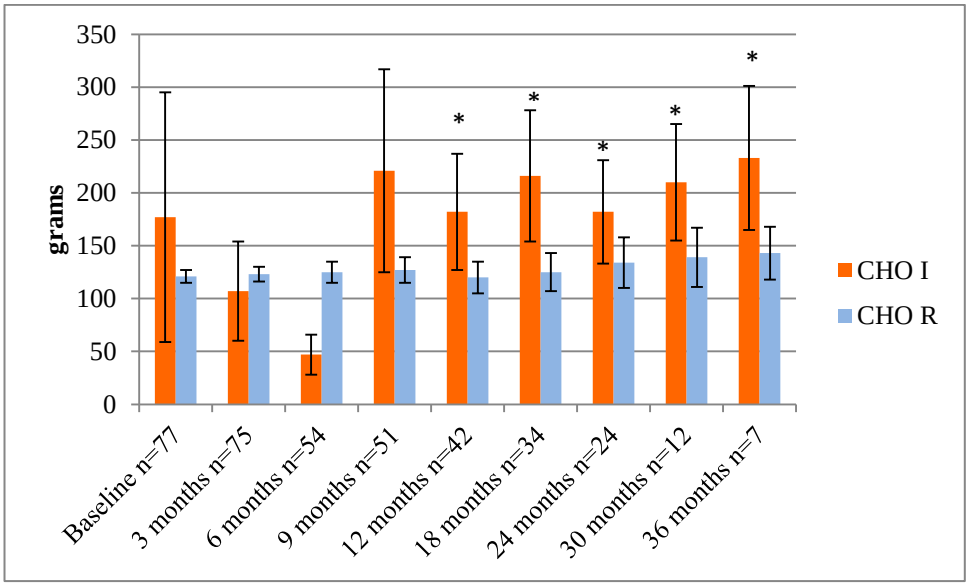

Figure 4.30 Carbohydrates intake (CHO I) compared with CHO recommendations (CHO R) at different stages of the disease

Recommendations presented in grams were calculated from percentage CHO recommendations of TEI: 40% for children <1 year and 50% for children >1year o age). Values are expressed as mean ± SD; *Indicate p<0.05 (Independent t-test).

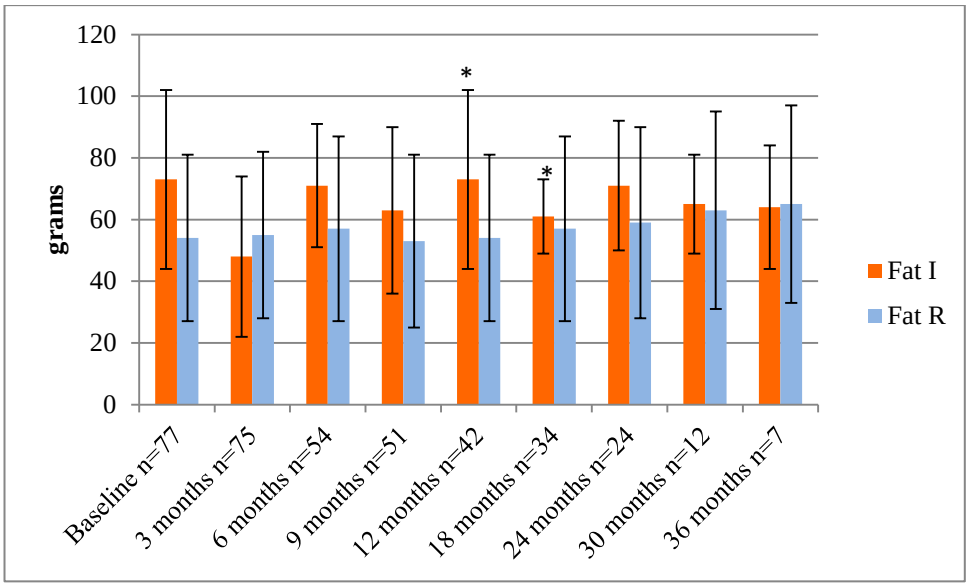

Figure 4.161 Fat intake (Fat I) compared with fat recommendations (Fat R) at different stages of the disease

Recommendations presented in grams were calculated from percentage fat recommendations of TEI: 50% for children <1 year and 35% for children >1year o age). Values are expressed as mean ± SD; * Indicate $p<0.05$ (Independent t-test).

4.3.8.2 Vitamin and mineral intakes

Vitamin and mineral intakes were obtained from 24 hour multiple pass dietary recall at different stages of the disease. Overall, mean vitamin intakes were above the RNI and mean mineral intakes were within the references ranges; however there was high variability between individuals for both vitamin and mineral intakes (tables 4.14 and 4.15).

Table 4-14 Vitamin intakes of paediatric cancer patients during the study period expressed as mean ± SD

	N	Vitamin A µg/day [1]RNI (350-700)	Vitamin E mg/day No [2]DRVs	Vitamin B12 µg/day RNI (0.3-1.5)	Vitamin C mg/day RNI (25-40)	Folate µg/day RNI (50-200)
Baseline	77	395±79	6.7±5.8	4.3±2.5	104±105	143±54
3 months	73	1659±2750	4.2±3.4	2.3±2.6	57±38	87±29
6 months	52	708±383	6.0±1.3	3.8±2.4	43±32	145±52
9 months	49	1470±1486	6.3±3.4	4.0±2.5	60±37	225±87
12 months	40	775±611	8.5±5.8	2.7±2.4	98±61	219±127
18 months	34	557±108	5.0±2.1	5.6±3.7	104±50	184±83
24 months	23	612±450	6.2±4.9	4.7±1.2	64±45	228±69
30 months	12	591±296	5.2±2.5	4.9±3.1	67±83	237±120
36 months	7	518±144	7.0±2.6	5.0±2.9	76±32	242±114

[1]RNI: reference nutrient intake between the ages of 0-18 years (Department of Health 1991); [2]DRVs: dietary reference values for Vitamin E are determined by PUFA intakes, no RNI has been established for vitamin E.

Table 4-15 Mineral intakes of paediatric cancer patients during the study period expressed as mean ± SD

	N	Copper mg/day Mean±SD [1]RNI (0.3-2.2)	Iron mg/day Mean±SD RNI (1.7-14.8)	Zinc mg/day Mean±SD RNI (4.0-9.5)	Selenium µg/day Mean±SD RNI (10-70)
Baseline	77	0.78±0.73	7.33±4.20	6.03±3.93	28.31±20.05
3 months	73	0.69±0.41	8.05±4.59	9.67±1.21	36.59±66.17
6 months	52	0.82±0.55	9.16±4.90	6.83±3.83	38.40±74.06
9 months	49	0.80±0.45	8.52±3.83	7.03±3.87	28.57±18.03
12 months	40	0.79±0.59	12.89±19.32	7.03±2.58	28.04±15.75
18 months	34	0.91±0.57	8.81±3.90	7.10±3.12	31.36±16.29
24 months	23	0.90±0.99	8.38±4.76	5.90±2.16	24.26±15.19
30 months	12	0.70±0.43	8.75±3.76	6.35±2.54	24.17±12.71
36 months	7	0.73±0.36	11.13±6.01	7.45±3.22	33.71±13.30

[1]RNI: reference nutrient intake between the ages of 0-18 years (Department of Health 1991).

4.3.8.3 Nutrition Support

A total of 55 (67%) patients were referred to the Dietitian for nutritional assessment during the study period. Of these, 50 (61%) were prescribed some form of nutritional support and 5 (6%) had general dietary advice. For further detail on specific type of nutritional support prescribed see table 4.16. The reasons for referral were: undernutrition/weight loss (16/55; 25%), reduced oral intake (10/55; 18%),

temporary gut failure (10/55; 18%), to prevent weight loss (7/55; 13%), dysphagia (4/55; 7%), steroid induced diabetes (2/55; 4%), mucositis (1/55; 2%) and following parent's request (1/55; 2%).

Total energy intakes (kcal/day) and macronutrient intakes (grams) of patients who were on nutritional support and those who were not were compared at every stage. Although patients who were not on nutritional support had consistently higher total energy intakes than those who were, the difference was not statistical significant at any stage. Additionally, there were no statistically significant differences in the overall (all stages of disease) BMI and HFA centiles, FM% and FFM% (estimated from arm anthropometry) between those who were on nutritional support and those who were not during the entire study period.

Table 4.17 describes how many undernourished children, who were identified by BMI and UAFA, and how many PEM children, who were identified by UAMA, were on nutritional support throughout the study period.

Table 4-16 Use of nutritional support according to diagnostic criteria

Diagnostic criteria	NS	Percentage	Type of nutritional support				
	N	%	ONS N(%)	NG N(%)	PEG N(%)	TPN N(%)	Advanced NS N(%)
Solid tumours	19/35	54%	2(6%)	9(26%)	2(6%)	0	7(20%)
Haematological Malignancies	23/36	64%	8(22%)	7(19%)	0	1(3%)	7(19%)
Brain tumours	5/7	71%	2(29%)	1(14%)	2(29%)	0	0
Others: LCH[1]	3/4	75%	2(50%)	0	0	0	1(25%)
Total[2]	50/82	61%	14(17%)	17(21%)	4(5%)	1(1%)	15(18%)

[1] Langerhams Cell Histiocytosis; [2]Some patients received more than one type of nutritional support

Table 4-17 Patients identified as undernourished or PEM and on nutritional support at different stages of the disease

	Undernourished BMI	[1]NS		PEM (UAMA)	NS		Undernourished (UAFA)	NS	
		N	%		N	%		N	%
Diagnosis	11	10	91	-	-	-	-	-	-
Baseline	11	7	64	3	3	100	9	4	44
3 months	5	5	100	6	3	50	4	3	75
6 months	4	1	25	4	2	50	2	2	100
9 months	1	0	0	3	1	33	1	1	100
12 months	0	0	0	3	2	67	2	2	100
18 months	0	0	0	1	1	100	1	1	100
24 months	1	1	100	3	3	100	3	3	100
30 months	0	0	0	-	0	-	0	0	-
36 months	0	0	0	-	0	-	0	0	-

[1]NS: Nutritional support.

4.3.9 Associations between nutritional status at diagnosis and clinical outcome

Associations between nutritional status, which was established by using BMI centile (undernutrition, well-nourished and overnutrition), at diagnosis and clinical outcomes were assessed. Only undernutrition was statistically significantly associated with event, which was defined as relapse, death or becoming palliative [Fisher's Exact test (19.901); $p<0.001$]. Additionally, patients who were undernourished at diagnosis were 14 times more likely to have an event (RR=14). However, when severity of disease was taken into consideration 67% of this group of patients were treated with a high risk protocol and 17% with either medium or low risk protocol. Finally, overnutrition (overweight and obesity) was not statistically significantly associated with clinical outcomes [Fisher Exact test (7.10); $p=0.3$].

4.4 DISCUSSION

This prospective cohort study from SE and E Scotland is the first and most comprehensive of its type as it investigates patterns of change and factors contributing to changes in nutritional status of paediatric cancer patients based on its full definition. This not only include changes in body size and body composition, but also any imbalance in total energy, macronutrients and micronutrients intake as well as plasma micronutrients. Additionally, plasma markers of stress as well as clinical outcomes were studied (BAPEN 2010).

4.4.1 Patients' characteristics

179 patients were diagnosed with paediatric cancer during the study period (Aug 2010-Jan 2014) in the RHSC, Edinburgh. This is a mean of 51 diagnoses per year, which is below the reported 55-60 mean diagnoses per year prior to Aug 2010. The recruitment rate of 81% (n=82 of 101 eligible patients) is slightly higher than the 78% reported in similar studies performed in Scotland (Reilly et al. 2001), similar to that reported in Central America 82% (Sala et al. 2008), but lower than that from other studies published in Central America (92%) (Antillon et al. 2008) and the Netherlands (100%) (Brinksma et al. 2014). An explanation for this could be the difference in the population included, such as differences in severity of disease, and either recruitment procedures and policies (opting out instead of opting in). The most common diagnoses in this study were the leukaemias (43%) with ALL being the most prevalent diagnosis (35%), followed by the lymphomas (10%) and then neuroblastoma and renal tumours equally (6%). The incidence of each type of cancer does not entirely reflect that of the UK (Cancer Research UK 2012a) or Scotland (Information Service Division Scotland 2011), which could be explained by a relatively small sample size and also by the fact that the National and Scottish records stratify the ranking of most common cancers by age groups: 0-14 years and 15-24 years (Cancer Research UK 2012a, Information Service Division Scotland 2011).

This study presented all diagnoses in one group (children aged less than 18 years). Age at diagnosis, gender, diagnostic criteria and deprivation (SES) as well as ethnicity of the patient's cohort were all representative of the population from SE

Scotland as shown by data from the control group. Additionally, gender ratio (boys: 56%; girls: 44%) was representative of both the UK (boys: 55%; girls: 45%) and the Scottish cancer population (boys: 56%; girls: 44%) (Cancer Research UK 2012a, Information Service Division Scotland 2011). Finally, the severity of disease was established by classifying patients into treatment risk due to the large numbers of protocols (n=24) used for treatment in this population (n=82). Of these, 22% (n=18) were classified as low risk, 37% (n=30) as medium risk and 41.5% (n=34) as high risk. At present, this information is not available nationwide, and most authors who report this information have only investigated children with ALL (Argüelles et al. 2000, Reilly et al. 2001, Antillón et al. 2008). Thus, this data can only be compared with findings from the Netherlands (Brinksma et al. 2014) who reported a higher proportion of children receiving moderate/medium (45%) and high risk treatment (48%), with a much smaller proportion receiving low intensity treatment than the population from this cohort.

This study showed a 90% survival rate by the end of the study period, which equates to the 1 year survival rates from Scotland (Information Service Division Scotland 2011). However, this study had a minimum follow up of 3 months and a maximum of 3 years, so comparisons with national data would not be appropriate.

4.4.2 Nutritional status of paediatric cancer patients

4.4.2.1 Prevalence of malnutrition and changes in nutritional status according to growth and body composition measurements

The first aim of this study was to investigate the prevalence of malnutrition and to identify patterns of change in the nutritional status of paediatric cancer patients at different stages of the disease and treatment. The emphasis on using a variety of methods (BMI, MUAC, TSF and BIA) to assess nutritional status was based on evidence which suggested that this was essential for the optimal nutritional management of this population (Viana et al. 1994, Anon.1998, Brennan et al. 1997, Sala et al. 2012, Reilly et al. 1999, Sala et al. 2005, Elhasid et al. 1999, van Eys 1979b, Donaldson et al. 1981, Garófolo et al. 2005, Carter et al. 1983, Brouwer et al. 2007, Anon.2010, Lobato-Mendizábal et al. 1989, González et al. 2004, Halton et al.

1998, Schiavetti et al. 2002, Tobias & Hochhauser 2010) and that the use of these methods allow for a more comprehensive assessment, as at present a single "*gold standard"* technique does not exist (Sala et al. 2005). Additionally, the results from recent systematic reviews (Brinksma et al 2012, Revuelta Iniesta et al. 2015) concluded that presently there is insufficient robust evidence to accurately determine the prevalence of malnutrition, especially during and at the end of treatment, and that there is a need for future high-quality population based longitudinal studies.

(i) Nutritional status according to growth

The results of this study showed that the prevalence of malnutrition varies between different type of cancers, measurements used and at different stages of the disease. However, there was not a gender difference.

Overall, undernutrition was more common at diagnosis and at baseline and ranged between 13-15% depending on the measurement used. This gradually reduced throughout treatment and by the 30 and 36 month follow up, no patient was classified as undernourished. Undernutrition was significantly associated with paediatric cancer at diagnosis, baseline and 3 months in comparison with expected prevalence of undernutrition from the healthy UK children and young people population (Department of Health 2012). However, this is lower than that reported in the otherwise healthy children from developing countries, which has been estimated to affect between 19-30% of children (Stephenson et al. 2000, de Onis et al. 2010). A recent systematic review reported a similar level (13%) of undernourished patients in other paediatric cohorts from the developed world (Revuelta Iniesta et al. 2015); although a lower prevalence has been reported in the Netherlands (8%) (Brinksma et al. 2014). As already highlighted however, until now malnutrition has not been investigated in a uniform or consistent way; thus making comparison of data problematic.

The overall higher prevalence of undernutrition seen at diagnosis in this study is not surprising and is likely to be multifactorial in orgin. This is often the result of tumour burden and the consequent increase in TEE before treatment (Schmid et al. 2005, den Broeder et al. 2001, Vaisman et al. 1993, Stallings et al. 1989), anorexia with a

decrease in dietary intake (Falconer et al. 1994, Staal-van den Brekel et al. 1995), and treatment induced side-effects from the initial intensive therapies (Brinksma et al. 2012, Antillón et al. 2008, Koskelo et al. 1990, (Pinkerton, Plowman and Pieters 2004, Bauer et al. 2011, Donaldson 1982, van Eys 1986, Donaldson et al. 1981).

In contrast, overnutrition and obesity rates were lowest at baseline ranging from 8-9% and 1-14% respectively. These are slightly lower than those reported in a recent systematic review (15%) (Revuelta Iniesta 2015), however this review considered overweight and obesity as overnutrition and reported values together. A much lower prevalence of obesity has been reported in the Netherlands (4.5%) in a study published after the systematic review was carried out (Brinksma et al 2014). This difference could be a reflection of the higher obesity prevalence among healthy children from the UK in comparison to that of the Netherlands (Bibiloni et al. 2013, Bates et al. 2011, Currie et al. 2012). Prevalence of both overweight and obesity increased steadily throughout the course of the disease reaching a peak at 36 months (14-28%) for overnutrition and 30 months (25-33%) for obesity. Importantly though only prevalence of obesity at 30 months was significantly associated with paediatric cancer when this was compared to expected obesity frequencies from the healthy UK population (Department of Health 2012). This data needs to be interpreted with caution owing to the small sample size at these later stages of the study (30 months, n=12; 36 months, n=7). Nevertheless, these finding are supported by many others worldwide, especially in survivors of childhood cancer (Inaba et al. 2012, Brinksma et al. 2014, Bibiloni et al. 2013, Oeffinger et al. 2003, Siviero-Miachon et al. 2009a, Reilly 2009a, Reilly 2009a, Iughetti and Bruzzi 2011, Aldhafiri et al. 2014, Showell et al. 2013, Taylor et al. 2013, Co-Reyes et al. 2012, Siviero-Miachon et al. 2009b).

Prevalence of malnutrition was stratified by type of cancer. Children diagnosed with solid tumours (range 17-22%), haematological malignancies (3-12%) and brain tumours (9-17%) had a significantly higher risk of being underweight at diagnosis and baseline than healthy children from the UK (Department of Health 2012). The highest prevalence of undernutrition at baseline was seen in the solid tumour group, ranging from 17-22% depending on the type of measurement used, whilst no child diagnosed with OAD presented undernourished. Higher prevalences of

undernutrition in children diagnosed with solid tumours have been reported in a recent systematic review (29.5%) (Revuelta Iniesta et al. 2015) where most studies reporting this data were from developed countries, mainly the USA, Canada and Europe. A lot of the populations from the studies, which looked at solid tumours and were included in the systematic review had severe disease; stage IV Neuroblastoma (Green et al. 2008), Wilms' tumour (Wessels et al. 1999), and Medulloblastoma as well as supratentorial PNET (Bakish et al. 2003), perhaps contributing to the higher prevalence in comparison to the findings from this study.

The prevalence of undernutrition seen in the haematological malignancy group at baseline is corroborated by those reported worldwide (13%) (Revuelta Iniesta et al. 2015) and in developed countries only (0-10%) (Brinksma et al. 2012). Although, haematological malignancies include a variety of diseases, such as ALL, AML or CML, most studies have either investigated children diagnosed with ALL only (Viana et al. 1994, Jaime-Pérez et al. 2008, Reilly et al. 1999, Gofman & Ducore 2009, Baillargeon et al. 2006, Baillargeon et al. 2007, Delbecque-Boussard et al. 1997, Antillon et al. 2008, González et al. 2004, Uderzo et al. 1996, Ahmed et al. 1997) or, like in this study, most of the population consist of ALL patients; consequently, contributing to the more consistent findings. In contrast, solid tumours include a wider spectrum of diseases in its classification, which could contribute to the high variability of results reported here and in other studies (Revuelta Iniesta 2015).

Only one study investigated children diagnosed with brain tumours reporting a higher prevalence of undernutrition (31%) (Bakish et al. 2003) than that obtained in this study. This discrepancy could be attributed to the difference between the type of cancers included in Bakish et al. (2003) (Medulloblastoma and supratentorial PNET) and this study (see table 4.2 and 4.3). The prevalence of undernutrition in all types of cancer was reduced during the treatment phase, especially after the 9 month follow up, and by the end of the study no child was undernourished. These findings contrast with those recently reported (Revuelta Iniesta et al. 2015) where prevalence of undernutrition during treatment were 23% and 14% and, at the end of therapy were

35% and 9.5% for the solid tumours and haematological malignancy group respectively.

The highest prevalence of overweight (0-25%) and obesity (0-50%) was found in children diagnosed with OAD, followed by those with brain tumours, in whom the prevalence was 0-28.5% for overweight and 17-28.5% for obesity at baseline. Surprisingly, children with solid tumours had a similar prevalence of overweight to those with haematological malignancies at baseline ranging from 0-9% and 5.5-9% respectively. However, obesity prevalence was higher in the haematological group (0-14%) compared to the solid tumours (0-8.5%). Although, a few studies have investigated overnutrition in brain tumours (Müller et al. 2001, Müller et al. 2011) at the time of diagnosis, this is the first study to report prevalence of overnutrition and obesity in both brain tumours and solid tumours, and also the first to report prevalence of malnutrition in children diagnosed with OAD (LCH) (Revuelta Iniesta et al. 2015, Brinksma et al. 2012) at this stage. Therefore, these results cannot be compared with other published studies. Overnutrition has been investigated more extensively in children diagnosed with haematological malignancies, especially at the time of diagnosis (Jaime-Pérez et al. 2008, Reilly et al. 1999, Gofman and Ducore 2009, Baillargeon et al. 2005, Baillargeon et al. 2007, Odame et al. 1994) and in survivors of ALL (Wallace et al. 2013, Dalton et al. 2003, Reilly 2009a, Iughetti and Bruzzi 2011, Tylavsky et al. 2010) and, the overall overnutrition prevalence at diagnosis of 15% found worldwide is comparable with the results obtained in this study (Revuelta Iniesta et al. 2015).

Overweight and obesity prevalence increased during the course of treatment in all type of cancers, especially in children diagnosed with haematological malignancies and brain tumours (table 4.5). Moreover, they were significantly associated with cancer when compared to expected overweight and obesity frequencies from healthy UK children and young people (Department of Health 2012). This increase in obesity seen in haematological malignancies is consistent with findings from a systematic review, which reported prevalence of overnutrition as high as 40% both during treatment and after the completion of therapy (Revuelta Iniesta et al. 2015). In contrast, those reported in the solid tumour group in this same systematic review are

higher than those obtained in this study: 21% during treatment and 35% after the completion of therapy (Revuelta Iniesta et al. 2015). Once more, caution must be taken at interpreting these results as sample sizes for each individual type of cancer were very small, especially at the later stages of treatment, and either underestimation or overestimations of malnutrition might have occurred.

Prevalence of malnutrition was stratified by gender. There was not a significant difference between males and females at any stage of the disease, and both genders were significantly associated with undernutrition at baseline and 3 months when this was compared against expected frequencies from the healthy UK children and young people (Department of Health 2012). Only male gender was associated with overweight and obesity at the end of the study period in compared to healthy UK children and young people (Department of Health 2012). Comparison between these findings and others is difficult because this study did not stratify the gender and type of cancer further due small sample size, especially at the end of the study. It is worth noting however that in other studies the prevalence of overnutrition in boys and girls diagnosed with ALL did not differ at diagnosis (Odame et al. 1994 and Reilly et al. 1999); however these and other UK (Warner et al. 1995) and USA authors (Oeffinger et al. 2003) reported higher frequencies of overnutrition in girls compared to boys at later stages of the disease and after treatment in children diagnosed with paediatric cancer and ALL respectively, which differs from the current study. Only one study from the UK reported no difference between girls and boys in overnutrition prevalence in survivors of ALL (Didi et al. 1995).

Despite the small sample size, this study highlights an overall high prevalence of undernutrition during the initiation of treatment with an increase in overweight and obesity at later stages. Additionally, children with solid tumours showed the highest prevalence of undernutrition during the initial phases of treatment, whilst those diagnosed with brain tumours and haematological malignancies exhibited higher prevalence of obesity after the completion of therapy. Finally, this study did not show a gender difference in the prevalence of malnutrition during the study period. The importance of these findings lies in the building of an epidemiological picture of

the nutritional status of paediatric cancer patients, which can be used to guide the future nutritional management of this population.

(ii) Nutritional status according to body composition

Protein energy malnutrition (PEM), established by using upper arm muscle area (UAMA) reference data (Frisancho 1981), was more prevalent at 3 months (10%) and, surprisingly, at 24 months (12.5%) than at baseline (5%). However, by the end of the study period no child was diagnosed with PEM. Malnutrition was established by using upper arm fat area (UAFA) reference data (Frisancho 1981), which identified 13.5% of undernourished children at baseline. This gradually reduced throughout the study period and by the end no child was identified as undernourished.

Children with solid tumours had a significantly lower UAMA when compared to other types of cancer; yet UAFA did not statistically differ between diagnoses at any stage. Likewise, UAMA and UAFA did not statistically differ between genders. Comparison of these findings with other studies is difficult due to the paucity of evidence looking specifically at PEM in this population, the different methods (and reference values) used to assess this, and the population being small and mainly of ALL children. Additionally, none of the studies investigating FFM and FM report the actual prevalence of PEM, but instead the patterns of change in body composition. Despite these drawbacks similar findings have been reported. Halton et al. (1998) assessed body composition using DEXA scan in 16 out of 116 ALL children included in the study. The author reported a decrease in FFM of 5% at 6 months and an increase in FM of 6% by the end of therapy (Halton et al. 1998). Similarly, Koskelo et al. (1990) in a prospective cohort study (controls v cohort), in which 12 ALL and 2 acute non-lymphocytic leukaemic children were included, assessed body composition using ultrasound method (US) and arm as well thigh measurements (MUAC, TSF, thigh circumference and thigh skinfold). This study showed that muscle mass was lower in the cancer group compared to the control at diagnosis and that muscle mass decreased by 27% following 4-6 weeks of treatment (Koskelo et al. 1990). Simultaneously, FM increased by 33% and body water also increased

(oedema), thus maintaining net body weight (Koskelo et al. 1990). Unfortunately, the initial percentage of FFM and FM was not reported.

Contrary to our findings, Murphy et al. (2010) in a cross-sectional study investigating the body composition of 48 children treated for cancer or haemophagocytic syndrome and 48 sex-matched controls found no difference in FFM, assessed by air displacement plethysmography, between the groups. Of note, the study did not state the length of treatment, which could explain the difference with the other studies. Interestingly, body mass cell assessed by total body potassium showed lower body cell mass in the cancer group than the control reflecting lower body function (Murphy et al. 2010). Like in the current study, FM was also higher in the cancer group (Murphy et al. 2010). Finally, Delbecque Boussard et al. (1997) in a prospective cohort study (cohort v control) of 15 ALL children showed no difference between the cohort and the controls in FFM and this was regardless of the measurement used, BIA or TSF. However, FFM reduced from a mean±SD of 20.8±6.8 Kg at day 1 to 15.6±5.5 Kg at day 22 of treatment (Delbecque-Boussard et al. 1997). The study did not report significance, probably due to the small sample size; however a mean reduction of 5.2Kg in FFM could reflect severe PEM.

Reasons for the high prevalence of PEM and undernutrition (assessed by UAFA), especially seen in the solid tumour group from the current study, could partly be attributed to the inflammatory response. This is supported by the high ssCRP, especially in solid tumours, high ferritin and hypoalbunaemia results seen in this study. It is well acknowledged that inflammation induces anorexia, which in turn leads to a reduction of dietary intake, skeletal muscle catabolism, which leads to muscle wasting, and to a lesser extent lipolysis, which may lead to reduced FM (Picton 1998, Bauer et al. 2011). This appeared to be the case in most undernourished patients from this study, especially during the first year of treatment; however, this study did not show that all children with PEM were also undernourished when this was assessed using either BMI or UAFA. In fact most children classified as PEM were identified as either well-nourished or even overnourished by BMI and UAFA, indicating a higher FM percentage, which may have masked PEM. This combination of PEM and “high” FM could be attributed to

the faster repletion of lean mass in comparison with fat mass caused by the inflammatory process and the cancer treatment. Also, the high TEI seen in this population could have led to an increase in FM instead of FFM. Although this study failed to assess physical activity using an accelerometer due to the difficulties encountered by the children and their families, a recent review reported a reduction in physical activity during the treatment phase in paediatric cancer patients (Winter et al. 2010), which may have also contributed to the reduced FFM and higher FM seen in this cohort of paediatric cancer patients.

In summary, this study showed a high prevalence of PEM with either undernutrition or overnutrition as established by BMI, especially during the initial 3 months of treatment. Considering the short and long term implications of PEM described in both healthy children and in paediatric cancer patients in chapter I (section 1.2.3) and in chapter III (section 3.1) (Viana et al. 1994, Cornelio-Nieto 2007, Sala et al. 2012, Sala et al. 2008, Mejía-Aranguré et al. 1999, Hoorweg & Stanfield 1976), and the discrepancy between BMI, UAMA and UAFA at identifying undernutrition and PEM, this study highlights the importance of assessing body composition alongside measurements of body size as part of regular nutritional monitoring in clinical practice.

(iii) Changes in body size and body composition during the study period

The short and medium term changes in nutritional status were established using BMI centiles, arm anthropometry and BIA, which allowed a comprehensive assessment of body size and body composition.

A number of findings are likely to be pertinent to both future research and clinical practice, and may be useful in patient's counselling. This study showed high individual variability in BMI centiles from diagnosis until the end of therapy indicating the heterogeneity of this population, which may be due to the different diagnosis, treatments, age and small sample size (of each individual diagnosis). Nevertheless, BMI centile increased overall throughout the course of treatment. Children diagnosed with solid tumours had consistently the lowest median BMI centiles compared to other diagnoses; whilst those diagnosed with OAD, brain

tumours and haematological malignancies had the highest median BMI centiles. Recent data from the Netherlands are consistent with these findings, albeit an overall increase in BMI centile in all type of diagnosis (Brinksma et al. 2014). Of note, this study, unlike the current study, did not investigate children diagnosed with OAD and the follow up period was 12 months (Brinksma et al. 2014), consequently comparison was limited to this period of time. There is a general consensus regarding the increase in either BMI or weight centiles in paediatric cancer patients from the time of diagnosis until the end of therepay; however most evidence comes from retrospective studies investigating children treated for haematological malignancies (Reilly et al. 2001, Gofman and Ducore 2009, Baillargeon et al. 2005, Baillargeon et al. 2007, Odame et al. 1994, Withycombe et al. 2009, Esbenshade et al. 2011) or survivors of ALL (Zhang et al. 2014, Baillargeon et al. 2005, Baillargeon et al. 2006, Oeffinger et al. 2003, Asner et al. 2008, Chow et al. 2007, Davies et al. 1995) with very long intervals of follow up (>6 months). Although, these studies are very informative at establishing trends, prevalence and possible causes of obesity in haematological malignancy patients and survivors, it is difficult to establish at what stage of the treatment obesity occurs and thus when to start targeting these patients with diet and lifestyle therapy.

The current study also revealed some interesting results when the data was stratified by treatment risk and age at diagnosis. Children classified as high risk, regardless of their diagnosis, had consistently the lowest BMI centiles, whilst those who were classified as low risk had the highest median BMI centiles; however by the end of the study period BMI centiles were similar in all treatment risk groups. Furthermore the BMI of children diagnosed at the age of ≥12 years increased dramatically from ~55th centile at diagnosis to ~95th centile by the end of the study, whilst those diagnosed between the ages of 2-11 and 0-1.99 years only increased 5 centiles during the study period, from 55th to 60th and from 35th to 40th respectively.

The lower BMI centiles from the high treatment risk group found in the current study is not surprising as the use of more anti-cancer treatments as well as their increased in intensity are likely to increase the number and severity of side-effects, such as mucositis, vomiting and malbsorption; consequently increasing the risk of

undernutrition (van Eys 1979a, Bauer et al. 2011, Donaldson 1982, van Eys 1986). Most of the published data are in line with the results from the current study, whereby children diagnosed with both advanced solid tumours (Israels et al. 2010, Green et al. 2008) and ALL treated with high risk protocols had lower BMI centiles, and thus were at higher risk of having undernutrition during the initial phases of treatment (Delbecque-Boussard et al. 1997, Halton et al. 1998, Mejía-Arangure et al. 1997, Esbenshade et al. 2011, Corera Sánchez et al. 1992). For instance, a case control study performed in México, in which 17 ALL children with severe undernutrition died during the initial phases of treatment (induction-to-remission phase) were compared with 76 ALL controls who had survived the initial phases of treatment. Although, the main aim of this study was to assess whether undernutrition was associated with an increased risk of mortality, the study also reported that children treated for high risk ALL with more intensive therapies had higher rates of undernutrition and lower BMI centiles (Mejía-Arangure et al. 1997). Likewise, a prospective cohort study (with healthy controls) performed in Canada, in which the weight for height of 12 children diagnosed with stage IV neuroblastoma and treated with a high risk protocol were investigated (Green et al. 2008). A total of 50% were undernourished at diagnosis, 30% during phase 2 of treatment and 20% in phase 3, which is in line with the patterns found in the current study. Unfortunately, the authors did not report weight for heights from the healthy controls, thus comparisons between cases and controls were not established (Green et al. 2008).

In contrast, a prospective cohort study performed in Canada, in which the weight and body composition of 116 ALL children were investigated reported that children treated for high risk ALL had higher weight centiles than those treated for standard risk ALL; however at 24 and 36 months of treatment their weight was the lowest (Halton et al. 1998). This also contrasts with the findings from the current study, as it was found that BMI centiles were similar by the end of therapy. These discrepancies could be attributed to the difference in treatment and diagnoses as the high risk group from the Canadian study were treated with cranial irradiation, which is associated with obesity (Armstrong et al. 2010, Iughetti et al. 2012), and they all had an ALL

diagnosis (Halton et al. 1998); whilst in the current study all type of diagnoses were included in the stratification of treatment risk due to the small sample size.

Evidence regarding the relationship between age at diagnosis and BMI trends are inconsistent, especially with regards to obesity. In contrast to the findings from this study, most evidence support a higher increase in BMI in younger children diagnosed with craniopharyngioma (Lustig et al. 2003) and ALL (Reilly et al. 2001, Oeffinger et al. 2003, Didi et al. 1995, Garmey et al. 2008), which has been attributed to an early adiposity rebound that predisposes to obesity in later life (Reilly et al. 2001, Lustig et al. 2003), and unhealthy behaviours (like sedentary lifestyle and limited food choices) adopted early in life as result of the long lasting treatment and their side-effects (Bernstein 1978, Bernstein et al. 1982, Reilly 2009a, Stolley et al. 2010, Winter et al. 2010, San Juan et al. 2011). In line with the findings from the current study, a retrospective cohort study, in which the nutritional status of 1451 survivors of childhood ALL and 2167 siblings were investigated in the USA (Garmey et al. 2008). It was found that ALL survivors had a greater rate of BMI increase, which started during the treatment phase, than their siblings. Additionally, older children and young people had a higher increase in BMI than younger children. Of note, it was reported that younger children who were exposed to CRT had a higher increase of becoming obese. This suggests that one of the differences between the current study and the published data might be the CRT treatment. No child from the current study received CRT as at present it is limited to children with refractory ALL and with optical nerve infiltration (Sposto 2004).

One of the most striking findings from this study is the short to medium term changes in body composition. MUAC remained constant throughout the study period; however TSF increased dramatically at 3 months, and then decreased slightly at 6 months, but it increased gradually thereafter. Furthermore, estimation of body composition using both arm anthropometry and BIA showed a gradual reduction in FFM% throughout the study period (from 69% to 63.5%), which is indicative of progressive muscle wasting due to inflammation and perhaps sedentary behaviour. In contrast, there was a gradual increase in FM% (from 30% to 36.5%) demonstrating an overall increase in fat mass. No significant changes in FFM% and FM% were

found at any stage of the study. Therefore, this study suggest that the body composition of childhood cancer patients starts changing gradually during the initial phases of treatment and remain until the end of therapy (3 years), which emphasises the need for measuring body composition alongside BMI as a routine clinical parameter. This would allow establishing short and long term patterns of change as one single measurement of body composition in this population would be clinically irrelevant.

These findings are consistent with most published data investigating arm anthropometry and body composition of ALL patients (Siimes et al. 1991, Halton et al. 1998, van der Sluis et al. 2002, Koskelo et al. 1990) and solid tumours during treatment (Skolin et al. 1997, Murphy et al. 2010, Taskinen et al. 2007, de Graaf et al. 1987). The aetiology of the changes seen in body composition may relate partly to the effects of the initial intensive chemotherapy phases combined with an inflammatory response (high CRP and ferritin and reduced albumin levels) seen in this study, thus leading to muscle wasting. This is further corroborated by an increase in fat mass, as excessive energy intake in catabolic individuals lead to fat deposition without an increase in muscle mass (Halton et al. 1998, Murphy et al. 2010). Previous studies have also shown that the use of glucocorticoids (Reilly et al. 2001) combined with an increase in TEE and reduced physical activity also contribute to an increase in FM during treatment (Brinksma et al. 2014, Halton et al. 1998, Murphy et al. 2010), which then continues into adulthood (Inaba et al. 2012, Zhang et al. 2014, Taylor et al. 2013). The follow up from this study was 3 years; consequently it is not known whether high FM% will remain in the long term.

It is noteworthy that the body composition is presented as a percentage of the total FFM and FM and that when only one is affected (reduced) the other one is automatically increased. However, this study showed both a decrease in FFM and an increase in FM as MUAC remained constant and TSF increased throughout the study period.

4.4.2.2 Linear Growth

The current study found that poor linear growth was prevalent in this cohort of paediatric cancer patients; however there appeared to be catch-up growth following 2 years of treatment. Poor linear growth was most pronounced in the haematological malignancy group, who showed a median reduction of 23 centiles following 2 years of treatment. A consensus exists regarding the poor linear growth of haematological malignancy patients, especially those diagnosed with ALL (Bond et al. 1992, Murphy et al. 2006, Halton et al. 1998, Ahmed et al. 1997, van der Sluis et al. 2002, Caruso Nicoletti et al. 1993, Viana and Vilela 2008), but also in children diagnosed with solid tumours (Bond et al. 1992, Murphy et al. 2006, Revuelta Iniesta et al. 2015). The factors contributing to poor linear growth have been attributed to cranial irradiation, poly-chemotherapy, younger age at diagnosis and growth hormone deficiency (Viana & Vilela 2008). Like this study Viana et al. (2008) did not find any statistical significant difference in the HFA of males and females. In fact, more evidence attribute poor linear growth to the increasing use of high dose poly-chemotherapy protocols and the long-term treatments, particularly in haematological malignancies, whose average treatment is 2 years for the females and 3 years for the males (Mycroft 2010). Linear growth is a complex mechanism, which is affected by chemotherapy, radiotherapy and HSCT as well as nutritional status (van Leeuwen et al. 2000). Particularly, the effects of corticosteroids on bone mass density have been well described. Corticosteroids inhibit growth hormone, the proliferation of osteoblast and reduce the secretion of sex hormones in adults, which in turn leads to an increase in bone resorption and a reduction of bone mass density (van Leeuwen et al. 2000). In periods of rapid growth in children, where the incident of childhood cancer is highest (3-4 years and puberty), corticosteroids do not only affect bone mass density, but also reduce bone growth velocity, leading to poor growth (El-Hajj Fuleihan et al. 2012, van Leeuwen et al. 2000, Kang and Lim 2013, Al-Jaouni et al. 2009). Additionally, the most commonly used chemotherapy agents; alkylating agents (cyclophosphamide) and antimetabolites (cisplatin and methotrexate) are also thought to inhibit longitudinal growth (Davies et al. 2005). The role of chondrocyte cells is essential for bone growth, however *in vitro* studies have found that these agents directly interfere in their life cycle, resulting in a decrease in bone growth and

consequently linear growth (van Leeuwen et al. 2000). Methotrexate has been found to suppress osteoblast activity and stimulate osteoclasts, resulting in bone resortion and poor growth (van Leeuwen et al. 2000). Finally, one of the known side-effects of HSCT is poor growth and reduced final HFA (Leiper 2002b, Leiper 2002a). Three children from this cohort received HSCT, and of these 2 had a reduction in HFA following HSCT and 1 did not have any further follow up.

This study showed catch up growth 2 and half years post-treatment, especially in the haematological malignancies. Although, this is consistent with some published studies (Murphy et al. 2006, Halton et al. 1998, Ahmed et al. 1997, van der Sluis et al. 2002, Caruso Nicoletti et al. 1993, Holm et al. 1994), caution should be taken at interpreting this data as at this stage there were only 12 children measured, which is unlikely to represent the whole cohort and most evidence investigating the final height of childhood cancer survivors report this to be reduced when this is compared to expected final height (Dalton et al. 2003, Müller et al. 1998, Sklar et al. 1993).

In summary, there was an overall reduction in HFA in the whole cohort of paediatric cancer patients, which was more prominent in haematological malignancies. This is likely to be the result of poly-therapy, especially chemotherapy and HSCT, as poor linear growth starts to become apparent soon after the start of treatment.

4.4.3 Factors contributing to nutritional status and patterns of change of paediatric cancer patients

This is the first prospective cohort study performed in Scotland, which has established patterns of change in body size (BMI and HFA centiles) and body composition (FFM and FM) in paediatric cancer patiuents following 18 months of treatment. Only one factor has been identified which significantly contributed to changes in nutritional status. This was treatment risk and it only exerted this influence during the first 3 months of treatment. No other factor assessed in this study (age at diagnosis, type of cancer, treatment risk, nutritional support and TEI) contributed to changes in nutritional status, established by BMI.

BMI and fat mass, measured with arm anthropometry and BIA, increased throughout the 18 months and both reached significance at 3 months. Interestingly, BMI had a

second significant increment at 18 months, whilst FM attained significance at 9 months instead. In contrast, FFM declined significantly at 3 and 9 months when this was assessed using BIA. Of note, significant changes in FM and FFM assessed using arm anthropometry were only established at 3 months. During this initial period of increase in BMI and FM, HFA centiles decreased significantly. These findings are analogous to a recent prospective cohort study, similar in design to the current study, published in the Netherlands (Brinksma et al. 2014). Like Brinksma et al. (2014), the current study highlights that changes in body size and body composition occurred early on following treatment in all paediatric cancer patients; however the current study also indicates that both the increasing trend of BMI and FM and the reducing trend in FFM remained after one year, for 18 months. Although this study was unable to establish trends following 18 months of treatment, there is evidence elsewhere which suggests that these changes continue into adulthood (Zhang et al. 2014, Siviero-Miachon et al. 2009a, Brouwer et al. 2007, Asner et al. 2008, Iughetti et al. 2012, Garmey et al. 2008). The consequences of reduced FFM during treatment include poor linear growth (Brennan 1998) loss of muscle strength, reduced tolerance of therapy and increase treatment induced side-effects as well as an increase in the risk of infections (Sala et al. 2012, Murphy et al. 2010). Furthermore, reduced FFM accompanied by high BMI and FM post-treatment increases the risk of cardiovascular disease even in later life more in this population (Wallace et al. 2013, Blijdorp et al. 2012).

Total energy intake significantly contributed to overnutrition, established by BMI, when this was analysed independently of all the other variables. The fact that energy intake exceeded energy requirements in most patients regardless of whether they were receiving nutritional support and that 67% patients received some form of nutritional support, highlights the active policies currently in place in the Hematology and Oncology ward from both the RHSC of Edinburgh and Ninewells in Dundee to both prevent and treat undernutrition as all paediatric cancer patients durin the treatment phase are adivised to have a high energy dense diet. This might also explain the rapid increase in BMI, which occurred following an initial decline in those patients who were undernourished. However, this study proposes that the

recovery in BMI is due to an increase in FM rather than FFM, thus PEM may be masked by high FM.

Repeated cycles of weight loss and weight gain typically seen in dieting behaviours, called weight cycling, may eventually lead to obesity, especially in adelescents (Dulloo et al. 2012). This has been attributed to hyperpagia following a period of starvation, consequently leading to rapid weight gain (Dulloo et al. 2012). Therefore, it could be hypothesised that children receiving alternative intensive cycles of chemotherapy and corticosteroids experience changes in body weight as consequence of the side-effects and appetite respectively, which combined with sedentary behaviour, may eventually lead to excesive and rapid fat accumulation and obesity.

Therefore, it appears that current nutritional practices have become very successful at reducing undernutrition and its risk; however overnutrition has largely been overlooked and at present overnutrition during the treatment phases is not tackled. This could be attributed to current understaffing of Dietitians, which unables the team to deal with the high nutritional demands of this population having to prioratise undernutrition, and also the inexistence of specific clinical guidelines for the management of paediatric cancer obesity.

Current paediatric oncology dietetic practices in SE Scotland base their nutritional assessments on either weight for height or weight only (Sala et al. 2004, Paciarotti I 2013). Although, rapid weight loss is an indicator for acute undernutrition, this measurement alone does not estimate FFM and FM. Consequently these early changes in body composition are neglected. Furthermore, the equations used in current paediatric oncology clinical practice to estimate energy requirements in children take into consideration growth and a physical activity level (PAL) of 25th centile (Department of Health 1991, Henry 2005). The current study, like others (Bond et al. 1992, Murphy et al. 2006, Halton et al. 1998, Ahmed et al. 1997, van der Sluis et al. 2002, Caruso Nicoletti et al. 1993, Viana and Vilela 2008, Revuelta Iniesta et al. 2015), has demonstrated that there is a stagnation of linear growth during treatment, especially during the first 3 months of treatment. Moreover, there is published evidence of a sedentary lifestyle seen both during and after treatment

among patients (Reilly 2009a, Stolley et al. 2010, Winter et al. 2010, San Juan et al. 2011). These two factors might have contributed to an overestimation of TER, which in turn might have led to an excessive TEI through nutritional treatment and general nutritional advice. Based on the reduced physical activity levels of paediatric cancer patients reported by the literature (Reilly 2009a, Stolley et al. 2010, Winter et al. 2010, San Juan et al. 2011), the current study used a PAL of the 10th centile; however the TEE equations and the PAL used also accounts for linear growth, which might have contributed to a small overestimation of TER during the initial 3 months of treatment. Despite this, TEIs were still significantly higher than TER.

Treatment risk was the most important contributor to changes in BMI during the first 3 months of treatment in this cohort. High risk treatment protocols contributed to an increased risk of undernutrition, whilst low treatment risk protocol contributed to an increased risk of overnutrition. These findings have also been supported by others (van Eys 1979a, Bauer et al. 2011, Donaldson 1982, van Eys 1986). It is also noteworthy, that the age at diagnosis also contributed to malnutrition when this variable was analysed independently. Younger children were at higher risk of becoming undernourished and older children were at higher risk of becoming overnourished. As discussed in section 4.4.2.1, iii, these findings contrast with most published literature (Oeffinger et al. 2003, Reilly et al. 2001, Lustig et al. 2003, Didi et al. 1995, Garmey et al. 2008) and reasons for this have previously been discussed.

The findings from this study have several implications for both clinical practice and future research. With regards to clinical practice, this study underlines the need for monitoring not only growth but also body composition by using either anthropometry or BIA. By adopting these measures, the excessive accumulation of fat in the adipose tissue could be identified earlier and dietary advice including TEI could be tailored accordingly. Equally, the muscle wasting found to be prevalent, especially at 3 and 9 months would be detected. Whilst some degree of muscle wasting resulting from the initial acute phase response is inevitable (Guadagni & Biolo 2009), attempts should be made to minimise it at these critical stages of the disease. It has been suggested that this could be achieved by increasing total protein and introducing physical activity (Brinksma et al. 2014). This study attempted to assess physical activity,

however this proved impossible as it became too laborious for the patients and their families. Nonetheless, evidence supports early interventions to increase physical activity, especially when treatment induced side-effects have started to lessen (Brinksma et al. 2014). Finally, the vulnerability of children treated with high risk protocols should be targeted to prevent or reduce the risk of undernutrition at the early stages (initial 3 months); whislt careful matching of TER and TEI and close monitoring should be aimed for to avoid overfeeding.

4.4.4 Methods used to identify body size and body composition

The findings from this study are based on the methods, reference values and thresholds used to establish nutritional status already discussed in the methods section. In agreement with a recent systematic review (Revuelta Iniesta et al. 2015), this study showed poor agreement between BMI and both MUAC and TSF at identifying malnutrition in paediatric cancer patients. Arm anthropometry measurements classified consistently more children as undernourished and overnourished, whilst BMI identified higher prevalence of obesity when this was compared to TSF during the initial phases of therapy in all diagnostic criteria. Inaccuracies can arise from weight, height and particularly length measurements (Norton and Olds 1996); nevertheless the intra- and inter-TEM of the observers was minimal and unlikely to have affected the results. Some bias could have been derived from length measurements as its intra and inter-TEM was not established. Of note, the recommended BMI threshold for undernutrition in children aged between 2-18 years of ≤2.3rd centile (-2SD) used in this study (Bates et al. 2011, SACN 2011, SACN/RCPCH 2012) and others (Reilly et al. 1999, Pietsch and Ford 2000) could have contributed to this discrepancy. More rigorous thresholds of ≤5th centile (severe undernutrition) or even ≤15th centile (moderate undernutrition) have been suggested (Sala et al. 2004, Sala et al. 2012); however, further research should investigate the precision of this cut-off values against more sensitive measures of assessment, such as DEXA scan, before they are implemented in this population. It is more likely that the discrepancy between the measurements during the initial phases of therapy further reflects the limitations of using BMI as the sole method of assessment in

paediatric cancer patients as it does not account for changes in body composition, oedema and weight of tumour.

Some limitations have been associated with arm anthropometry measurements and the reference values used in this study. Firstly, high TEM, particularly between observers (inter-TEM), has been reported by many (Ulijaszek and Kerr 1999, Jamaiyah et al. 2010, Geeta et al. 2009, Ulijaszek and Lourie 1997). Nevertheless, the intra- and inter-TEM of TSF, weight and height was found to be minimal and unlikely to have contributed to the discrepancies seen in this study. Interestingly, the inter-TEM for MUAC was slightly higher and may have contributed to some error. Secondly, the estimation of body composition by arm anthropometry assumes that FM is equally distributed throughout the body (Norton and Olds 1996). This is certainly not the case in adults, but fat deposition in children (between 6-18 years) has been reported to be more equally distributed (Roche et al. 1981). Thirdly, the threshold used to identify undernutrition by arm anthropometry was $\leq 5^{th}$ centile. This reference value comes from a population of children from the USA in the 1970s, which can be assumed is different from the population in this cohort and thus underestimation of undernutrition and overestimation of overnutrition might have arisen. There is a clear urgent need to update MUAC and TSF reference values that includes all ages (0-18 years). Lastly, the difficulty of taking these measurements for research purposes in a population who is severely ill and already receiving many forms of invasive treatments should not be underestimated.

The main limitations of single-frequency BIA have been previously discussed (chapter I, section 1.6.2.1). In addition to these limitations and as a result of clinical practices, it was impossible to always measure the patients in the morning or at the same time, fasted and after micturating. Notwithstanding the limitations of the single-frequency BIA, the lack of reliable and specific TBW equations and the difficulties encountered with the reference values to assess body composition in paediatric cancer patients, this study revealed good limits of agreement between BIA and arm anthropometry for fat mass (1.2 to -1.0). This suggests that the use of BIA and/or arm anthropometry should be implemented in clinical practice. Other studies have investigated nutritional status in paediatric cancer patients using these two

different methods of assessment (Brennan et al. 1997, Murphy et al. 2009, Brinksma et al. 2014, Delbecque-Boussard et al. 1997, Oguz et al. 1999, Smith et al. 1991); however only one study compared the reliability of these two methods and found that BIA was more reliable due to the reduced inter and intra-TEM in compare to TSF (Brennan et al. 1997). Of note, a study in which adults on haemodialysis were included investigated the agreements between a single- and a multi-frequency BIA at assessing body composition. Although both methods showed good agreement, the multi-frequency BIA was more precise (Raimann et al. 2014). Findings which can be attributed to the functions of both types of BIA: multi-frequency BIA evaluates TBW and, unlike single-frequency BIA, distinguishes between intra- and extracellular fluid accounting for the rapid shifts in fluids of this population (Gudivaka et al. 1999, Kyle et al. 2004c, Kyle et al. 2004b). This would suggest that multi-frequency BIA is more appropriate for a similar population like the one from this cohort, nonetheless more research is needed to elucidate whether this is the case.

4.4.5 Biochemical and micronutrient blood parameters

The third objective of this study was to longitudinally investigate micronutrient status at different stages of the disease and to establish possible factors that may contribute to abnormalities. Biochemical markers were assessed to establish liver function, kidney function and the levels of inflammation. Abnormalities in any of these factors are likely to affect micronutrient status, and thus they will be considered altogether when interpreting the results.

Predictably, the current study showed compromised liver function, kidney function and signs of inflammation in many paediatric cancer patients. Acute liver injury was prevalent in all paediatric cancer patients, especially during the initial intensive treatment phase, and this remained throughout the study period in solid tumours and haematological malignancies. Overall, kidney injury was less prevalent than liver injury; however it was still predominant in solid tumours and haematological malignancies during the intial phases of treatment. Finally, there were signs of inflammation in all diagnostic criteria except brain tumours throughout the study period.

4.4.5.1 Antioxidant micronutrient status

This longitudinal study provides preliminary data on antioxidant micronutrient status in paediatric cancer patients. Whilst serum α-tocopherol:cholesterol ratio (vitamin E/Ch) was within the normal range in almost all patients, hypervitaminosis and hypovitaminosis A was prevalent in all type of cancers during the study period. Possible factors contributing to abnormal vitamin A levels were then explored. It was found that GGT, albumin, BMI centile and total vitamin A intake positively correlated with vitamin A status. Additionally CRP negatively correlated with plasma vitamin A. However, neither vitamin A supplementation nor ALT was significantly associated with vitamin A status.

Consideration of vitamin E/Ch results in view of the relevant literature is difficult because of the paucity of evidence. Furthermore, the few available studies have investigated vitamin E levels, lymphocytes or erythrocytes α-tocopherol, mainly in ALL patients (Battisti et al. 2008, Kennedy et al. 2004a, Misaki et al. 2003, Ghalaut et al. 1999, Singh et al. 2000), instead of vitamin E/Ch status in all type of paediatric cancers. Nevertheless, most data, unlike the current study, supports a decrease in vitamin E during treatment (Battisti et al. 2008, Kennedy et al. 2004a, Misaki et al. 2003), which returns to normal at the end of therapy (Battisti et al. 2008). It appeared that vitamin E/Ch status was not affected in this cohort perhaps due to an appropriate vitamin E intake during the study period, as vitamin E/Ch status was found to correlate with vitamin E intakes; however this is preliminary data and further studies should confirm these findings.

The findings from this study suggest that poor nutritional status, reduced vitamin A intake and inflammation might have contributed to plasma hypovitaminosis A. Hypovitaminosis A is well documented in undernourished children from developing countries as a result of reduced poor intake, which includes vitamin A (Akhtar et al. 2013, Laxmaiah et al. 2013) and, although very few studies have investigated plasma vitamin A in paediatric cancer patients (Kennedy et al. 2004a, Malvy et al. 1997, Fiore et al. 1997, Kennedy et al. 2005), consensus exists regarding a high prevalence of hypovitaminosis A, especially at diagnosis and during the initial phases of treatment, in this population. Two possible mechanisms by which hypovitaminosis A

occurred in this study are proposed. Firstly, vitamin A is transported in the blood by RBP and albumin and both of these proteins are synthesised in the liver. In an acute phase state, like inflammation, the synthesis of both proteins is downregulated, thus causing a reduction in plasma vitamin A (Blomhoff et al. 1991). Secondly, RBP is a small molecule, which is filtered in the kidneys (Blomhoff et al. 1991). In normall circumstances, it binds to transthyretin forming a complex that prevents the excretion of vitamin A. However, either moderate renal impairment or infection, very common in this group of patients, increases the permeability of the glomerulus, which may in turn result in significant vitamin A losses (Blomhoff et al. 1991, Penniston and Tanumihardjo 2006).

In contrast, it could be hypothesised that liver damage and high vitamin A intakes may have contributed to hypervitaminosis A (Blomhoff et al. 1991, Smith & Goodman 1976). Yet, this study did not show an association between supplementation and hypervitaminosis A. The high prevalence of hypervitaminosis A seen in this study is in line with Kennedy et al. (2005). But, unlike the findings from the current study, the authors did not establish an association between vitamin A intake and plasma levels. They attributed hypervitaminosis A to prednisone and hypothesised that this increases the synthesis of RBP leading to hypervitaminosis A (Kennedy et al. 2005). The current study did not investigate this association; however it is postulated that liver impairment may lead to both/either a reduction in hepatic vitamin A uptake and/or an increase in the release of stored vitamin A into the bloodstream, consequently causing plasma hypervitaminosis A (Smith & Goodman 1976).

The role of vitamin A in the body has extensively been studied in healthy individuals. As well as been an antioxidant, vitamin A participates in the process of vision helps protecting the skin from sunburn and it is essential for optimal growth and bone remodelling (Stahl & Sies 2012, Tanumihardjo 2013). More recently vitamin A, especially retinoic acid, has been linked with the regulation of more than 500 genes acting as a transcriptional activator or repressor of gene expression (McGrane 2007); however, neither the consequences of abnormal vitamin A levels in

paediatric cancer patients during cancer treatment nor whether this is part of the cancer and treatment process have been investigated.

4.4.5.2 Non-antioxidant micronutrient status

Plasma vitamin B12, folate and ferriting levels were considered at different stages of the disease throughout the study period. The results from this study showed that both plasma hyper and hypovitaminosis B12 were prevalent in all type of cancers, except in the brain tumours. Additionally, reduced serum folate was prevalent in all types of cancer, especially in the haematological malignancy and the solid tumour group. Finally, plasma ferritin levels were extremely high in the haematological malignancy and in the solid tumour group. These abnormalities were seen thoughout the study period despite vitamin B12 and folate mean intakes being above UK RNIs (Department of health 1991).

The high prevalence of hyper and hopovitaminosis B12 seen in this study is in line with three studies performed in adults diagnosed with solid tumours (Carmel and Eisenberg 1977), metastatic cancer (Carmel 1975) and multiple myeloma (Vlasveld 2003). The reasons for these findings are unknown, but it has been hypothesised that hypervitaminosis B12 could be caused by either an increased synthesis of vitamin B12-binding protein or a reduced clearance as a result of the malignancy and the chemotherapy (Carmel & Eisenberg 1977). Plasma hypovitaminosis B12 has been attributed to malabsorption and the long term use of proton pump inhibitors (PPI) (Force et al. 2003). However, this study does not support the latter as most patients received long term PPI treatment during the study period, but had different plasma vitamin B12 levels.

Contrary to the findings from this study, two studies performed in adults diagnosed with multiple myeloma (Vlasveld 2003) and solid tumours (Carmel & Eisenberg 1977) who were receiving treatment, reported plasma folate to be within the normal range. Paediatric cancer patients, especially those diagnosed and treated for haematological malignancies, received high doses of intrathecal methotrexate for their treatment regularly. One of the mechanisms of action of methotraxade is the inhibition of dihydropholate reductase, which converts dihydrofolate to the active

form of folate (tetrahydrofolate). Although, rescue treatment with folinic acid (leucovorin) is then administered, it could be postulated that serum folate levels do not recover completely in some patients despite appropriate folate intakes. Nonetheless, at present folic acid supplementation is discoraged due to the possible interactions with the chemotherapy and the proliferation of cancer cells as shown in adults with advanced colorectal cancer (Byström et al. 2009) and prostate cancer (Tomaszewski et al. 2011).

Certainly, more research should focus on investigating plasma vitamin B12 and folate and whether there are any associations with clinical outcomes. Specific mechanisms that affect the changes in plasma vitamins and minerals seen in this study are also warranted. Thus, at present, the only clinical recommendation that arises from this study is to continue monitoring the plasma micronutrients of paediatric cancer patients to provide a better epidemiological picture of this population and form the bases for future clinical trials.

The extremely high levels of serum ferritin seen particularly in the haematological malignancy, and also in the solid tumour group, is not only a reflection of an acute phase response, but also a possible suggestion of iron overload, which has been set at >1000 µg/L (Henter et al. 2007, Schrappe & Pieters 2004). The reasons for this have been attributed to an increase in the synthesis of ferritin by blast cells (cancer cells) and also a rare microphage activation syndrome, which is fulfilled by meeting five out of eight criteria (see chaper I, section 1.5.2) (Henter et al. 2007, Schrappe & Pieters 2004). Although, this study did not assess these parameters, it could be postulated that chelation iron therapy might be appropriate when ferritin levels reach >1000 µg/L; however intervention studies should be performed before this therapy is implemented.

4.4.6 Nutritional support

This longitudinal study aimed to identify the use of nutritional support throughout the study period. The results showed a high need for nutritional support (67%) in all type of cancers at any time point during the study period; this being most prescribed during the intial phases of treatment. OAD (75%; 3/4) and brain tumours (71%; 5/7)

had the highest prevalence of nutritional support with ONS being the most commonly used, which was 50% (2/4) for OAD and 29% (2/7) for brain tumours. In contrast, the solid tumours (54%; 19/35) and the haematological malignancies (64%; 23/36) required less nutritional prescriptions overall; however there was a higher need for NG [ST (26%; 9/35) and HM (19%; 7/36)] and advanced NS [ST (20%; 7/35) and HM (19%; 7/36)]. This indicates either a greater risk of undernutrition in these populations or that they had more severe disease and as a consequence had received more intensive treatment. Contrary to these findings, others (Brinksma et al. 2014, Paciarotti 2013) have reported a lower percentage of nutritional support required in paediatric cancer patients of 45% and 44% respectively. This could be attributed to the smaller sample size of this study (n=82) in compared to the others (n=133 and n=168 respectively). Different nutritional policies could have also contributed to the different findings as one study was performed in the Netherlands (Brinksma et al. 2014) and the other was retrospective, reflecting past and less aggressive nutritional policies which were implemented before the current study was performed (Paciarotti 2013).

Another aim of this study was to identify how many patients classified as undernourished or PEM were on nutritional support. Most PEM and undernourished patients received some form of nutritional support, however only 33% and 50% PEM patients (between 3 to 12 months) and 25% undernourished patients as per BMI (at 6 months) were on nutritional support. The fact that many PEM patients were not on nutritional support is not surprising as these were classified as normal weight, overweight or obese using BMI. Furthermore, it has been postulated that standard nutritional practices might not be beneficial to this population due to the further increase in fat mass instead of the desired muscle mass. Instead, they might benefit from higher protein intakes, reduced fat and carbohydrates in combination with patient-tailored physical activity (Zhang et al. 2014, Brinksma et al. 2014, Rickard et al. 1983). Nonetheless, this remains to be elucidated.

Future interventional studies investigating the efficacy of nutritional interventions on the nutritional status (body size and body composition) of paediatric cancer patients are needed. Different physical activity strategies should also be incorporated

alongside different amounts of macronutrients, especially protein. This combination would allow testing for both optimal protein intakes and different physical activity strategies that can either reduce or minimise muscle wasting during cancer treatment in this population.

4.4.7 Nutritional status at diagnosis as a prognostic factor for clinical outcomes

This study attempted to investigate whether nutritional status (undernutrition, overnutrition and obesity) at diagnosis could be used as a prognostic factor for clinical outcome, which was either survival or event (relapse, becoming palliative or death) of paediatric cancer patients. However, owing to the small sample size, it was impossible to perform a multivariate analysis. Instead a series of univariate analyses were performed as preliminary analysis of associations. The results suggested that undernutrition was significantly associated with an event. Moreover, this group was also 14 times more likely to have an event during the course of treatment. Interestingly, when severity of disease was also considered, it became apparent that most of these undernourished children were also receiving a high treatment risk protocol, suggesting that nutritional status is likely to be affected by severity of disease and treatment protocol. Therefore, children in high risk treatment protocols might be more likely to be undernourished at diagnosis and also to relapse, become palliative or to die. Most evidence from low income countries (Viana et al. 1994, Sala et al. 2012, Mejía-Arangure et al. 1997) and high income countries (Donaldson et al. 1981, Lobato-Mendizábal et al. 1989, Rickard et al. 1983) support these findings. Of note, Sala et al. (2012) did not find that undernourished children at diagnosis were more likely to relapse. Nonetheless, comparison of these results with the published literature needs to be interpreted with caution due to the difference in both the population and the available resources in each country (Pritchard-Jones et al. 2013). For instance, diagnoses in low income countries tend to be delayed due to reduced resources. As consequence, children present with more advanced diseases, a higher prevalence of undernutrition at diagnosis, which is often accompanied by more severe undernutrition, especially those children diagnosed with solid tumours (Israels et al. 2010, Israels et al. 2010, Green et al. 2008, Wessels et al. 1999).

In contrast, this preliminary analysis of associations suggested that obesity was not associated with clinical outcome (event). Comparison of these findings with published literature is difficult because the two published studies have investigated children diagnosed with ALL only, and both studies reported contradictory results (Butturini et al. 2007, Baillargeon et al. 2006). Even if obesity is not associated with an event (relapse, becoming palliative and higher mortality), it is essential to reduce its prevalence as obese children tend to have higher treatment induced side-effects, which often leads to delays in treatment and more admissions to hospital (Butturini et al. 2007). Unfortunately, this study did not look at these factors. It should also be noted that the results from the current study might have been affected by the statistical simplification performed, as "event" included three different clinical outcomes (relapse, death or becoming palliative). Undoubtedly, more research is needed to clarify whether nutritional status is associated with clinical outcome. This would allow paediatric oncologists to have more information to provide optimal nutritional treatment and to improve patient's care and their quality of life during treatment.

4.4.8 Limitations of study

Like many clinical studies, particularly involving paediatric cancer patients, there are some limitations which may apply to this study. Due to the nature of this longitudinal study, the time constrictions, and the large number of drop outs, the sample size was reduced during the later stages of the study. The reasons for the large number of drop outs were death, becoming palliative, re-allocation of treatment or missing data due to severe treatment induced side-effect. As a result, a mixed multilevel model to identify potential factors that contributed to changes in nutritional status and a multilevel growth model, which would have identified statistical signifant changes in nutritional status over time, was limited to 18 months instead of 36 months.

Ideally anthropometry and body composition measurements should always be taken at the same time to reduce patients' individual variability (Norton and Olds 1996, Pederson and Gore 1996). This was impossible due to clinical reasons, as the resaerchers were limited to already allocated appointments or other medical procedures. Furthermore, inaccuracies as a result of the use of sf-BIA are likely to

occur because some of the recommended conditions could not be met during the data collection period. For instance, it is recommended that patients are fasted and that their bladder is emptied prior to the measurement is taken. Also, measurements should be taken in the morning or always at the same time to reduced patients' individual variability, which was not always possible.

The body composition predictive equation used in this study has been developed from a healthy children population and then validated and modified accordingly in paediatric renal patients (Schaefer et al. 1994a, Schaefer et al. 2000). The authors reported good limits of agreement and coefficient of variation (%) with measurements obtained from total body potassium; however FFM tended to be overestimated by as much as 5.8% (Schaefer et al. 1994a), which could have had implications in the results of this study. Nonetheless, the FM% limits of agreement obtained from BIA and arm anthropometry were small (+1.2 to -1.0) suggesting accuracy of results.

The assessment of dietary intake using a 24 hour multiple pass recall may have underestimated or overestimated intakes in many patients, particularly when assessing micronutrient intakes in children who were not enterally or parenterally fed. Likewise, plasma vitamins and minerals was not always performed when the patients were fasted, which might have led to some unreliable results.

The reference ranges and cut-off values used to identify malnutrition, PEM and body composition in this study carry limitations themselves. It has been reported that current UK growth charts identify less undernourished and more obese children (SACN/RCPCH 2012) in comparison with the one used in this study (Cole et al. 1995a). If this were to be the case, this study would have overestimated undernutrition and underestimated obesity slightly; however this appears unlikely as measurements of TSF identified more undernourished children than BMI, especially during the initial phases of treatment. Furthermore, this study used the lowest recommended threshold of <2.3rd centile (-2.0 SD) for undernutrition (SACN 2011, SACN/RCPCH 2012), which has been widely used in the UK (Reilly et al. 1999, Pietsch and Ford 2000); however others recommend more rigorous cut off values

(Sala et al. 2012, WHO 1995) that are also able to establish different grades of undernutrition (moderate undernutrition <15th centile and severe undernutrition <5th centile). These thresholds might be more suitable for this paediatric cancer population, especially in those diagnosed with severe disease.

The reference ranges used for arm anthropometry could be argued that are now dated (Frisancho 1981, Frisancho 1974); however, it is the only one that offers a full reference for children between the ages of 1-18 or 0-18 years (Frisancho 1981, Frisancho 1974), instead of only focusing on specific age groups (WHO 2011). Thus allowing for more consistency, especially in this longitudinal cohort study where measurements have been taken over a long period of time. Finally, different reference ranges were used for children between 0-5 years (Fomon et al. 1982) and between 5-18 years (Wells et al. 2012). Therefore, inconsistences might have occurred in those children who were monitored during this time (4-5 years).

One of the major limitations of this study was the fact that the assessment of physical activity by means of an accelerometer during the treatment phase failed. This study showed that this was too challenging for the patients and their families. Future studies should attempt to use practical methods such as validated physical activity questionnaires, which are less accurate, but have been proved more successful for this paediatric oncology population (Brinksma et al. 2014).

4.5 CONCLUSION

This study has highlighted that children diagnosed and treated for cancer may be at high risk of undernutrition and PEM, particularly during the initial phases of treatment, and overnutrition, at later stages. It has also suggested that treatment risk may be an important factor contributing to undernutrition during the initial phases of treatment and no other clear factors assessed in this study contributed to malnutrition at later stages of the disease. This perhaps explains the high need for nutritional support seen in this population. Finally, the results emphasised the need for more epidemiological and mechanistic research, which investigates the plasma micronutrients status of paediatric cancer as many had an abnormal plasma micronutrient profile.

CHAPTER V

5. PLASMA VITAMIN D (25-HYDROXICHOLECALCIFEROL) LEVELS OF PAEDIATRIC CANCER PATIENTS IN SOUTH EAST SCOTLAND: A PROSPECTIVE COHORT STUDY

5.1 INTRODUCTION

Chapter II, section 2.2 showed that the prevalence of vitamin D deficiency and insufficiency in European paediatric cancer patients is 41% and 50% respectively, which is higher than that of healthy children and paediatric cancer patients from North America (15% and 46%) and the Middle East (24% and 51%) (Revuelta Iniesta et al. 2015b). Despite vitamin D inadequacy (deficiency and insufficiency) being an internationally recognised health problem (Ahmed et al. 2011) and the existence of vitamin D supplementation guidelines for children in the UK (RCPCH 2013, Holick et al. 2011), their implementation remains inconsistent (Pramyothin and Holick 2012).

As it has previously been discussed vitamin D is essential to allow optimal calcium homeostasis, and thus adequate growth and skeletal development in children (Holick et al. 2011). A potential role in lowering blood pressure, the risk of developing Diabetes Mellitus (Holick 2010) and a role in cancer prevention have also been reported (Holick and Chen 2008). Vitamin D is primarily absorbed from sunlight, but it can also be obtained from the diet. However, very few foods contain naturally occurring vitamin D (Holick 2006), and in the UK where fortification is discretionary, foods are rarely fortified with vitamin D (SACN 2007). Therefore, in high latitude countries, like Scotland, where UV-B rays are insufficient during the winter months (October to April) (SANC 2007), populations are at an increased risk of vitamin D deficiency and insufficiency. Other factors contributing to vitamin D inadequacy in healthy children have been attributed to skin pigmentation, obesity and age (infants and adolescents) (RCPCH 2013, Holick 2006).

Children diagnosed and treated for cancer tend to experience multiple side-effects, which might affect plasma 25(OH) D. These include phototoxicity, which requires children to avoid direct sunlight, malabsorption and reduced dietary intake (Oeffinger et al. 2006a), and hepatotoxicity and nephrotoxicity both of which may interfere with the activation of vitamin D (Zhou et al. 2006). Prolonged periods of vitamin D inadequacy in children increases the risk of bone fractures, rickets and of slow growth (Herbst et al. 2006), with a subsequent increased risk of osteoporosis in later life (Oeffinger et al. 2006b). Most children and adolescents treated for cancer

now survive into adulthood (Wallace et al. 2013); however they have an increased risk of developing the metabolic syndrome, cardiac complications and have a reduced peak bone mass, as well as developing secondary malignancies as a consequences of treatment (Wallace et al. 2013). Despite the emerging evidence of the importance of vitamin D on health, the high reported prevalence of vitamin D inadequacy in Europe and the recent call for high-quality population-based longitudinal cohort studies, there are few published studies in the UK and none in Scotland, investigating plasma 25 (OH)D levels in paediatric cancer patients (Revuelta Iniesta et al. 2015b). To address this clinical question a study with the following aims was designed:

(i) To investigate both plasma 25(OH)D and parathyroid hormone (PTH) levels of paediatric cancer patients at different stages of their disease

(ii) To compare plasma 25(OH)D levels of healthy children with a paediatric cancer cohort from Scotland

(iii) To explore possible factors (age, ethnicity, gender, seasonality, nutritional status, diagnosis, treatment and the use of nutritional support) contributing to plasma 25(OH) D inadequacy at baseline, 3 and 6 months.

5.2 METHODS

5.2.1 Study design, population and time-line

A prospective cohort study was performed. The eligibility criteria were: (i) children aged <18 years; (ii) diagnosed with cancer (ICCC-3) (Steliarova-Foucher et al. 2005) or Langerhans Cell Histiocytosis; (iii) diagnosed between Aug-2010 to Jan-2014 (iv) attending the South East Scotland regional center for Haematology and Oncology at the Royal Hospital for Sick Children (RHSC), Edinburgh or Ninewells Hospital, Dundee (Paediatric Oncology Shared Care Unit). The researchers excluded children who were treated palliative at the time of diagnosis and stopped monitoring those who became palliative during the study period, out of respect for the patients and their families. Children were monitored for a maximum period of 3 years and measurements were obtained at baseline (newly diagnosed patients), 3, 6, 9 and 12 months and every 6 months thereafter.

Control data were obtained from a case-control study of Vitamin D in children with epilepsy compared to controls performed at the same time as this study. Controls were randomly recruited from the RHSC Emergency Department and only healthy children without acute or chronic conditions were included in the study. Controls were seen at baseline and then asked to return six months after to obtain summer and winter samples in each. Where controls did not return for their follow up blood sampling, additional controls were recruited to obtain the needed number of samples for that season. No advice on vitamin D supplementation was provided until after the second appointment.

5.2.2 Demographics and clinical parameters

Clinical data (diagnosis, treatment protocol, treatment risk (low, medium and high) (Kazak et al. 2012) and length of treatment) and demographic data (age, gender, ethnicity and socio-economic deprivation) were collected from the medical notes. As a proxy marker for socioeconomic deprivation level of individuals, the individual residence postcodes was used to assess deprivation level of areas of residence using the Standard Index of Multiple Deprivation (SIMD) [The Scottish Government, 2012]. SIMD is presented as a quintile where "I" denotes the most deprived and "V" the least deprived.

The paediatric cancer cohort was grouped according to the wider definition of solid tumours, haematological cancers, brain tumours and other associated diagnoses.

5.2.3 Data collection

Plasma 25(OH) D, parathyroid hormone (PTH), calcium, phosphate and magnesium levels of the paediatric cancer cohort and the controls were measured. For assays details see appendix VII. Plasma 25(OH) D from the patients was analysed using the liquid chromatography tandem mass spectrometry technique by the Clinical Chemistry Department, Royal Infirmary of Glasgow using standard techniques. The intermediate coefficient of variation (%) of the latter assay was ≤8.9%. Plasma 25(OH) D from the controls was measured in Freeman Laboratoties, Newcastle using a competitive immunoassay chemiluminescence technology, which had a coefficient of variation (%) of ≤8.1%. All 25(OH)D results were expressed as nmol/L.

The analysis of calcium, phosphate and magnesium were all performed in the Edinburgh RHSC. The methods and equipment/kits used were the following: calcium (Gosling 1986, Kessler and Wolfman 1964), phosphate (Daly and Ertingshausen 1972) and magnesium (Elin 1987) and Abbott Architect C8000 was used for all of them. PTH was analysed in the Edinburgh Royal Infirmary accredited clinical laboratory. The method changed in August 2013. The first equipment used was Intact PTH Architect system (8K25) and PTH was expressed as pg/ml and the second and current one is IMMULITE 2000 Intact PTH SIEMENS (PIEL2KPP-5(19)) and results are expressed as pmol/L. All plasma PTH results obtained before August 2013 were converted from pg/ml to pmol/L. For more detailed on how these parameters were measured see appendix VII.

Plasma 25(OH) D levels were classified as summer months (1st of April-30th Sep) or winter months (1st Oct-31st Mar). Owing to the reduced number of healthy controls at follow up, the mean plasma 25(OH) D results of the summer and winter samples were calculated. The single winter or summer sample was used for those who missed the follow up appointment. This was then used for statistical analyses. Plasma 25(OH) D was defined according to the Royal College of Paediatrics and Child health (2013); suboptimal (50-75nmol/L), insufficient (25-50nmol/L) and deficient (<25nmol/L). Inadequacy was defined as <50nmol/L. The PTH reference value used was 1.7-7.5pmol/L (RHSC Edinburgh Clinical Chemistry Laboratory Handbook 2014) (appendix V).

Height (or length) and weight were measured using standard procedures by two trained researchers (RRI, IP). BMI centile was calculated and UK BMI growth centiles were used. Nutritional status was classified as underweight (BMI ≤2.3th centile) and overweight (BMI ≥85^{th}) (Cole et al. 1995a). Vitamin D intake was assessed by two trained researchers (RRI, IP) using a 24 hour multi-pass recall method and consequently analysed in WinDiets® (Univation Ltd 2005) (Wise 2005). Information on nutritional treatment and vitamin D supplementation was also recorded. Nutritional treatment was prescribed according to Subjective Global

assessment by the multidisciplinary team and consisted of enteral +/- parenteral nutrition (macronutrient) and micronutrient (vitamin D according to UK RCPCH guidelines (RCPCH 2013) or multivitamins), and a combination of macronutrients and micronutrients. Therapeutic vitamin D supplementation was stopped once patients achieved optimal plasma 25(OH) D levels.

This study was granted ethical approval from NHS Scotland (NHS REC 06-51104-52) and all patients' data were anonymised and kept confidential.

5.2.4 Statistical analyses

The Statistical Package for Social Science (IBM-SPSS for Windows Statistics, version 19, state and country) was employed to analyse all data. Descriptive statistics were used to evaluate the prevalence of plasma 25(OH)D inadequacy and comparisons between the paediatric cancer cohort and the healthy controls at diagnosis were performed using the Mann-Whitney test (aim i); associations between plasma 25(OH)D and the following variables; calcium, PTH, BMI centile and age, were performed using Spearman's correlation (aim ii and iii). Univariate associations between demographic data and categorical variables were established by $\chi 2$-test (aims ii and iii). $P < 0.05$ was considered statistically significant. The STROBE guidelines for the presentation of the data was followed (www.strobe-statement.org).

5.3 RESULTS

5.3.1 Demographic and clinical characteristics

A flow diagram of the paediatric cancer cohort is presented in figure 5.1. Thirty-three (out of 35) healthy controls and 65 (out of 82) paediatric cancer patients had plasma 25(OH) D samples available at baseline.

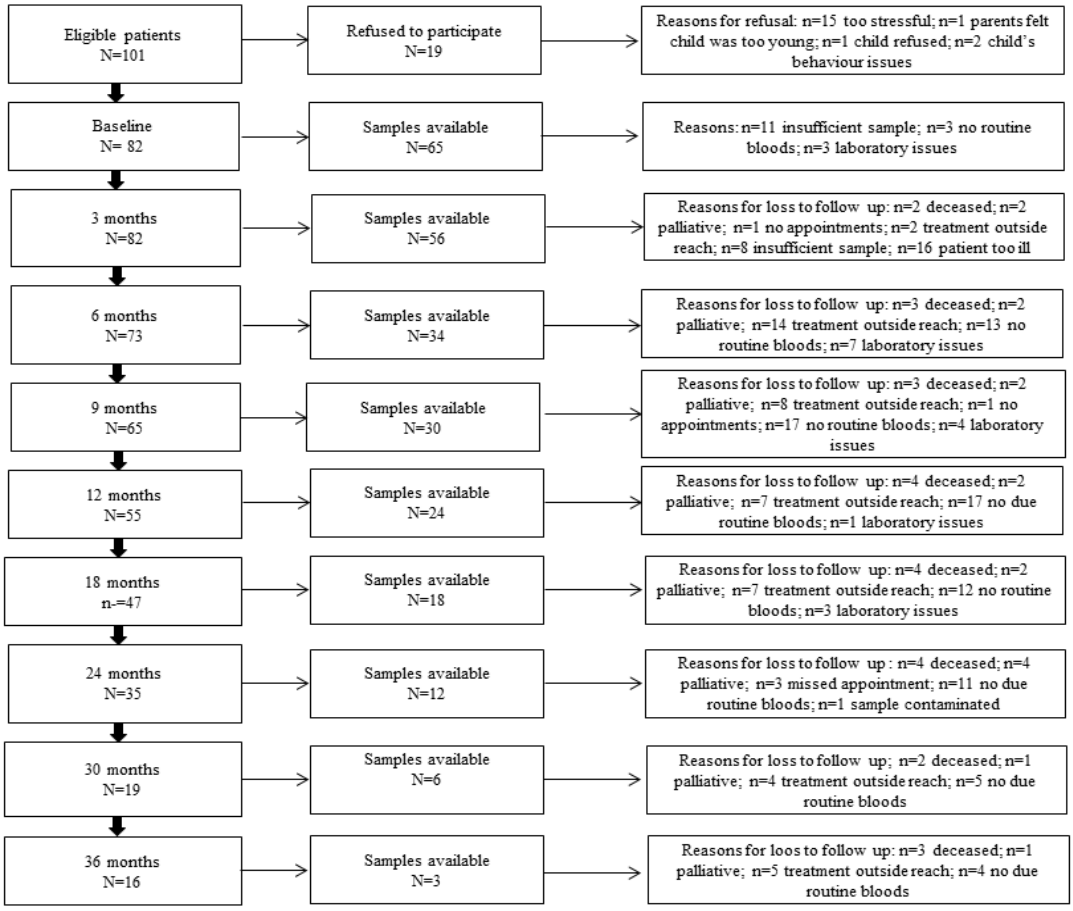

Figure 5.1 Flow chart showing the sample size at different stages of the study period

The demographic and clinical characteristics of the population are presented in table 5.1 and table 5.2. Gender, ethnicity and socioeconomic status as well as age at diagnosis did not statistically differ between the controls and the paediatric cancer cohort. BMI centiles were significantly lower in the paediatric cancer cohort. Twenty-four treatment protocols were used to treat the paediatric cancer cohort, the

median (IQR) time follow up was 312 (123.5-653.2) days and 22% (n=18) were classified as low risk, 37% (n=30) as medium risk and 41.5% (n=34) as high risk. The time between diagnosis and baseline measurements was 15.5 (10.0-25.0) days. Baseline plasma 25(OH)D of the paediatric cancer cohort did not statistically differ from the healthy controls (p=0.7).

Table 5-1 Characteristics of paediatric cancer patients and the healthy controls

Baseline characteristics	**Paediatric cancer cohort**		**Controls**		**P value**
Total sample (n)	82		35		
Age median (IQR)	3.9 (1.9-8.8)		6.2 (4.8-9.1)		0.1[1]
BMI centile median (IQR)	50 (19.0-84.5)		60.5 (43.7-89.5)		0.003[1]
Vitamin D median (IQR)	38.0 (21.0-61.0)		37.5 (23.0-58.0)		0.7[1]
	n	%	n	%	
Gender					0.5[2]
male	46	56.1	17	48.6	
female	36	43.9	18	51.4	
Ethnicity					0.6[2]
White	80	97.6	33	94.3	
Non-white	2	2.4	2	5.7	
SES					0.06[2]
I	15	18.3	3	8.6	
II	13	15.8	8	22.9	
III	15	18.3	5	14.3	
IV	24	29.3	5	14.3	
V	15	18.3	14	40.0	
Haematological malignancies	**35**	**43**	-	-	
ALL	29	35			
AML	3	4			
CML	2	2			
HLH	1	1			
Solid tumours	**39**	**47**			
Lymphomas	10	12			
Neuroblastoma	6	7	-	-	
Retinoblastoma	2	2	-	-	
Renal tumours	6	7	-	-	
Hepatic tumours	1	1	-	-	
Malignant bone tumours	4	5	-	-	
Soft tissue sarcoma	5	6	-	-	
Germ cell tumours	1	1	-	-	
Malignant epithelial neoplasm	4	5	-	-	
Others unspecified malignancy	0	0	-	-	
Other associated diagnoses-LCH	**3**	**4**	-	-	
Brain tumours-CNS tumours	**5**	**6**			

ALL: acute lymphoblastic leukaemia; AML: acute myeloid leukaemia; CML: chronic myeloid leukaemia; HLH: haemophagocytic lymphohistiocytosis; LCH: Langerham's cell Histiocytosis; CNS: central nervous system; [1]Mann-Whitney test; [2]chi square-test.

Table 5-2 Plasma 25(OH)D levels of the controls and the paediatric cancer cohort

		N	Median (IQR)	Deficient N (%)	Insufficient N (%)	Suboptimal N (%)	Optimal N (%)	P value
controls		n=35	37.5 (23.0-58.0)	8 (22.8)	14 (40.0)	6 (17.1%)	5 (14.3)	0.06[1]
Paediatric cancer	**Cases**	n=65	38.0 (21.0-61.0)	19 (29.2)	23 (35.4)	16 (24.6)	7 (10.8)	0.01[1]
	Diagnostic group	ST n=32	35.0 (16.0-60.0)	12 (37.5)	10 (31.2)	8 (25.0)	2 (6.25)	0.02[1]
		HM n=26	38.0 (27.7-52.2)	5 (19.2)	12 (46.1)	6 (23.1)	3 (11.5)	0.04[1]
		BT n=5	69 (14.5-75.5)	2 (40.0)	0	2 (40.0)	1 (20.0)	-
		OAD n=2	80	0	0	1 (50.0)	1 (50.0)	-
	Nutrition Support	None n=44	34.0(20.2-52.7)	16 (36.4)	15 (34.1)	11 (25.0)	3 (6.9)	0.006[1]
		Macronutrients n=14	43.0(29.2-75.7)	2 (14.3)	6 (42.8)	3 (21.4)	3 (21.4)	0.8[2]
		Micronutrients n=7	71.0(41.0-97.0)	1 (14.3)	1 (14.3)	3 (42.9)	2 (28.6)	0.9[2]

ST: Solid tumours; HM: Haematological malignancies; BT: Brain tumours; OAD: other associated-diagnoses; [1]χ^2-test; [2]Fisher's Exact test.

5.3.2 Plasma 25(OH)D levels

At baseline, of the 82 paediatric cancer patients, 17 (21%) did not have plasma 25(OH)D available due to clinical reasons (figure 5.1), 34 (41%) were summer and 31 (38%) were winter samples. There was no statistical significant difference [U (453); p=0.3] between the summer (median 39.0, IQR 30.0-62.0) and winter (median 36, IQR 16.0-61.0) plasma 25(OH)D levels of the paediatric cancer cohort at baseline and throughout the study period, apart from the 3 month follow up (figure 6.2). Of the 35 controls, 17 (49%) were winter only samples, 10 (28%) summer only samples and 6 (17%) provided repeated samples (winter and summer). Two (6%) samples were never returned due to laboratory issues. There was a statistically significant difference [U (42.5); p=0.003)] between winter (median 26.0, IQR 18.0-46.5) and summer plasma 25(OH)D (nmol/L) (median 56.5, IQR 45.5-78.0) levels in the control group.

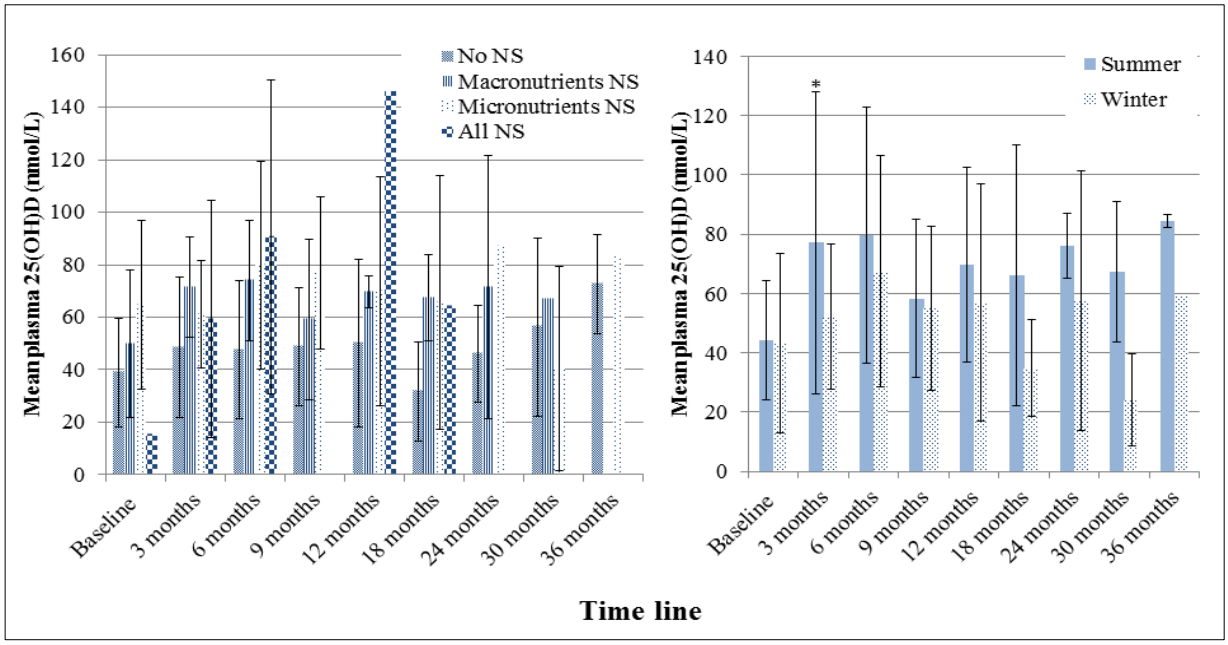

Figure 5.2. Plasma 25(OH)D with data stratified according to type of nutritional support (left) and by seasonal variation (right)

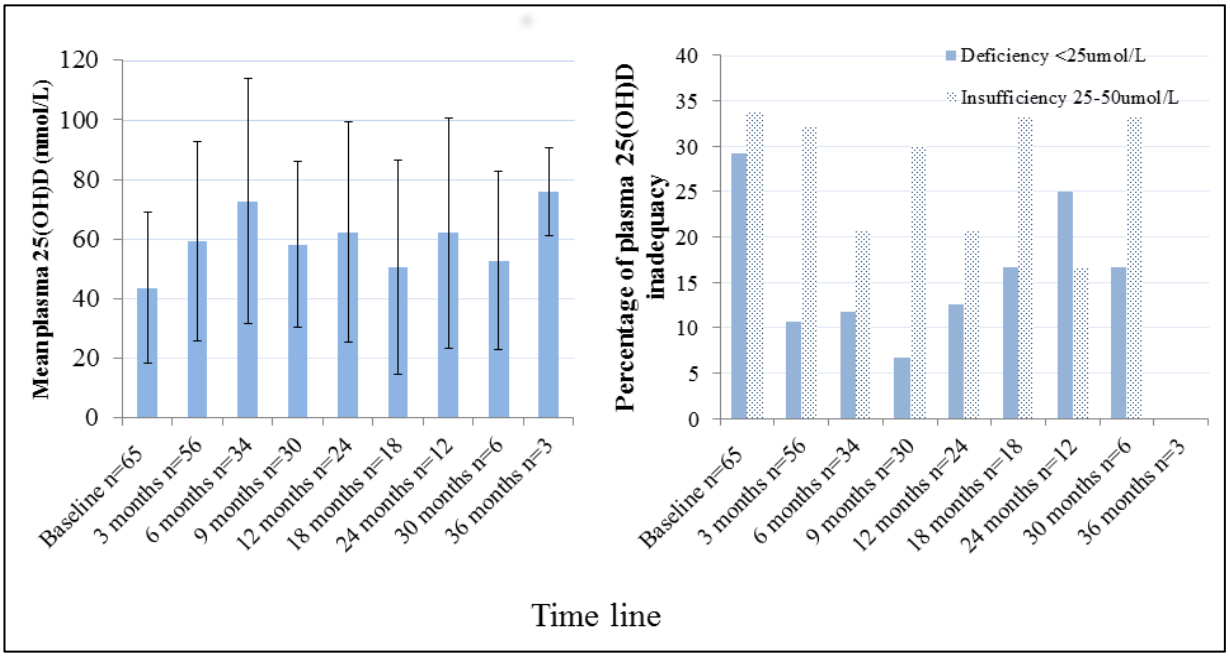

Figure 5.3 Plasma 25(OH)D levels (left) and prevalence of 25(OH)D deficiency and insufficiency (right) at different stages of the study period

The prevalence of plasma 25(OH)D inadequacy (deficiency and insufficiency) was 64% (42/65) in paediatric cancer patients and 63% (22/35) in healthy children at

baseline. However, there was a higher prevalence of plasma 25(OH)D deficiency in paediatric cancer patients (n=19; 29%) in comparison with healthy children (n=8; 22%). In the paediatric cancer cohort, the prevalence of plasma 25(OH)D inadequacy ranged between 33-50% throughout the study period and did not significantly change at any stage (figure 5.3). Stratification of the data by type of tumour showed that patients with solid tumours had the highest prevalence of 25(OH)D inadequacy (34%) followed by haematological malignancies (26%), both at baseline (table 5.2) and during the course of treatment (table 5.3).

Table 5-3 Prevalence of plasma 25(OH)D inadequacy with data stratified by diagnostic criteria

Time line	Plasma 25(OH)D levels	Solid tumours		Haematological malignancies		Brain tumours		OAD[1]	
		N=32	100%	N=26	100%	N=5	100%	N=2	100%
Baseline	Deficiency	12	37.5	5	19.2	2	40.0	0	0
	Insufficiency	10	31.3	12	46.2	0	0	0	0
	Suboptimal	8	25.0	6	23.1	2	40.0	1	50.0
		N=28	100%	N=25	100%	N=7	100%	N=2	100%
3 months	Deficiency	1	3.6	5	20.0	0	0	1	33.3
	Insufficiency	10	35.7	7	28.0	0	0	0	0
	Suboptimal	7	25.0	9	36.0	1	14.3	0	0
		N=14	100%	N=15	100%	N=2	100%	N=3	100%
6 months	Deficiency	1	7.1	2	13.3	0	0	0	0
	Insufficiency	5	35.7	1	6.7	1	50.0	0	0
	Suboptimal	2	14.3	7	46.7	0	0	2	100
		N=6	100%	N=13	100%	N=3	100%	N=2	100%
12 months	Deficiency	1	16.7	2	15.4	0	0	0	0
	Insufficiency	4	66.7	4	30.8	1	33.3	0	0
	Suboptimal	1	16.7	3	23.1	1	33.3	2	100
		N=4	100%	N=12	100%	N=1	100%	N=1	100%
18 months	Deficiency	0	0	2	16.7	0	0	1	100
	Insufficiency	2	50.0	4	33.3	0	0	0	0
	Suboptimal	1	25.0	4	33.3	1	100	0	0

Deficiency: <25µmol/L; Insufficiency: 25-50µmol/L; suboptimal: 50-75µmol/L; [1]OAD: other associated diagnoses.

Nutritional support was prescribed to 26% (21/82) of paediatric cancer patients at baseline. Of those on nutritional support, 14/82 (17%) were on macronutrient (enteral +/- parenteral nutrition), and 7/82 (8%) were receiving both macronutrient and micronutrient. The median (IQR) time between the start of nutritional support and

baseline was 8 (0-23) days. Eighty percent (66/82) of paediatric cancer patients received one or more forms of nutritional support for several days or weeks during the study period. Of these, 39/82 received macronutrient supplementation only, which provided between 0.7µg (28IU) vitamin D/100 Kcal to 1.9µg (76IU) vitamin D/100 Kcal, 48/82 (58%) received both micronutrient and macronutrient supplementation containing vitamin D, which provided between 10µg/day (400IU) to 500µg (20.000IU) single dose of vitamin D, and 21/82 (26%) received macronutrient only and micronutrient (+/- macronutrient) supplementation at different times. The median (IQR) vitamin D intake from diet alone was 1.2 (0.3-2.4) µg.

Figure 5.2 shows that paediatric cancer patients who were not supplemented had the lowest plasma 25(OH)D throughout the study period. The prevalence of plasma 25(OH)D inadequacy with data stratified by type of nutritional support and at different stages of the disease and treatment is presented in table 5.4. This was highest in those children who did not receive supplementation with median (IQR) ranging from 32.0 (21.0-46.5) nmol/L at 18 months to 45.0 (28.0-64.5) nmol/L at 24 months. In contrast, paediatric cancer patients supplemented with micronutrient (+/- macronutrient) followed by macronutrient alone had the lowest prevalence of plasma 25(OH)D inadequacy and the highest plasma 25(OH)D at most stages of the disease; the median (IQR) plasma 25(OH)D ranged from 63.0 (42.7-128.5) nmol/L at 6 months to 83.0 nmol/L at 36 months and from 43.0 (29.2-75.7) nmol/L at baseline to 79.0 (49.0-93.0) nmol/L at 6 months. Importantly, a considerable number of patients in the macronutrient subgroup had already received micronutrient supplementation. Of the 7 patients who were on macronutrient support at 6 months, all of them had already received micronutrient supplementation previously. Likewise, 2/5 (40%) patients on macronutrient support at 12 months and 1/2 (50%) patients at 18 months had received micronutrient supplementation in the previous follow up. Moreover, micronutrient supplementation was significantly associated with lower prevalence of plasma 25(OH)D inadequacy (Fisher's Exact test; p=0.04; RR 0.27; 95% CI 0.04-1.8) at 6 months. Three patients reached plasma 25(OH)D toxicity levels (>170 nmol/L) following high dose (500 µg/day) of vitamin D.

Plasma 25(OH)D did not significantly correlate with plasma calcium, phosphate, magnesium and PTH at any stage in the paediatric cancer cohort, however there was a significant correlation between PTH and plasma 25(OH)D levels [r=0.6 (strong); p<0.001] in the healthy controls. Table 6.5 shows PTH levels stratified by plasma 25(OH)D status at different stages of the disease.

5.3.3 Factors contributing to 25(OH)D inadequacy levels at baseline and during treatment

Age was significantly correlated with plasma 25(OH)D levels in paediatric cancer patients [R=-0.46 (moderate and negative); p<0.001], only at baseline, and in healthy children [R=-0.42 (moderate and negative); p<0.02], whereby older children had lower plasma 25(OH)D levels. Although, BMI centile was not significantly correlated with plasma 25(OH)D levels in the paediatric cancer cohort at baseline [R=-0.2 (weak and negative); p=0.08], 3 months [r=-0.2 (weak and negative); p=0.2] and 6 months [r=-0.2 (weak and negative); p=0.3], and in the healthy control [R=-0.3 (weak and negative); p=0.3], overnourished paediatric cancer patients were significantly more likely to have higher prevalence of plasma 25(OH)D inadequacy [χ^2-test(8.3); df(1); p=0.005; RR 3.1; 95% CI 1.4-14.0] at 3 months than healthy and undernourished children with cancer, regardless of whether the patients were on nutritional supplementation. Non-supplemented children were more likely to be deficient or insufficient (RR 4.3; 95% CI 1.1-4.7) at 6 months (Fisher's Exact test; p=0.04) in comparison with those who were supplemented with micronutrients.

None of the following categorical variables were significantly associated with plasma 25(OH)D status and paediatric cancer patients at any stage; treatment risk, diagnostic criteria, ethnicity and gender.

Table 5-4 Plasma 25(OH)D levels stratified by type of nutritional support

Deficiency: <25nmol/L; Insufficiency: 25-50nmol/L Suboptimal: 50-75nmol/L; Optimal: >75

Time line	Nutritional support		Deficiency		Insufficiency		Suboptimal		Optimal		Median (IQR)
		N	N	%	N	%	N	%	N	%	
Baseline N=65	None	44	16	25	15	23	11	17	2	3	34.0(20.2-52.7)
	Macronutrients	14	2	3	6	9	3	5	3	5	43.0(29.2-75.7)
	Micronutrients+/- macronutrients	7	1	1	1	1	3	5	2	3	71.0(41.0-97.0)
3 months N=55	None	25	4	7	11	20	6	11	4	7	45.0(32.0-56.0)
	Macronutrients	9	1	2	1	2	4	7	3	5	67.0(48.0-76.5)
	Micronutrients+/- macronutrients*	20	1	2	6	11	7	13	6	11	67.5(38.0-87.7)
6 months N=34	None	9	3	9	2	6	4	12	0	0	45.0(16.5-68.0)
	Macronutrients	7	0	0	2	6	1	3	4	12	79.0(49.0-93.0)
	Micronutrients+/- macronutrients	18	1	3	3	9	4	12	10	29	78.0(49.2-134.5)
9 months N=30	None	16	1	3	7	23	6	20	2	7	45.0(31.0-61.0)
	Macronutrients	8	1	3	2	7	2	7	3	10	59.0(35.5-84.25)
	Micronutrients+/- macronutrients	6	0	0	0		2	7	4	13	77.5(66.5-101.0)
12 months N=24	None	11	3	12	4	17	1	4	3	12	36.0(24.5-79.0)
	Macronutrients	5	0	0	1	4	4	17	0	0	63.0(51.0-63.0)
	Micronutrients+/- macronutrients	8	0	0	0	0	5	21	3	12	63.0(57.0-128.5)
18 months N=18	None	12	3	17	7	39	2	11	0	0	32.0(21-46.5)
	Macronutrients	2	0	0	0	0	1	6	1	6	67.5(56.0-)
	Micronutrients+/- macronutrients **	3	0	0	0	0	3	17	0	0	64.0(42.7-134.0)
24 months N=12	None	5	1	8	2	17	2	17	0	0	45.0 (28.0-64.5)
	Macronutrients	0	0	0	0	0	0	0	0	0	
	Micronutrients+/- macronutrients	7	2	17	1	8	0	0	4	33	67.0(23.7-106.2)
30 months N=6	None	3	1	17	2	33	0	0	0	0	35.0(13.0-)
	Macronutrients	1	0	0	0	0	1	17	0	0	67.0
	Micronutrients+/- macronutrients	2		0	0	0	1	17	1	17	82.0(68.0-)
36 months N=3	None	1	0	0	0	0	1	33	0	0	72.5(59.0-)
	Macronutrients	0	0	0	0	0	0	0	0	0	
	Micronutrients+/- macronutrients	1	0	0	0	0	0	0	1	33	83.0

<170nmol/L; *n=2 vitamin D > 170nmol/L; **n=1 vitamin D >170nmol/L

Table 5-5 Pathyroid hormone levels and status with data stratified by plasma 25(OH)D status

Time line	Plasma 25(OH)D levels	Deficiency <25nmol/L			Insufficiency 25-50nmol/L			Suboptimal 50-75nmol/L			Optimal >75 <175nmol/L		
		N	%	Median (IQR)	N	%	Median (IQR)	N	%	Median (IQR)	N	%	Median (IQR)
Baseline	↓PTH	1	5.9	3.2	1	5	3.3	3	17.6	4.0	1	16.7	2.6
N=60	Normal PTH	13		(2.4-7.3)	18	90	(2.6-4.3)	12	70.6	(1.9-5.1)	5	83.3	(2.0-4.1)
	↑PTH	3			1	5		2	11.8		0		
3 months	↓PTH	0	0	3.2	2	13.3	2.9	0		2.8	3	20	2.6
N=49	Normal PTH	6	100	(1.7-4.6)	12	8.0	(1.9-4.2)	13	100	(2.3-3.6)	10	66.6	(1.9-5.9)
	↑PTH	0	0		1	6.7		0			2	13.3	
6 months	↓PTH	0		2.7	0		3.2	0		2.6	4	28.6	2.1
N=34	Normal PTH	4	100	(2.7-3.5)	7	100	(2.5-4.9)	8	88.9	(2.0-5.3)	9	64.3	(1.3-4.0)
	↑PTH	0			0			1	11.1		1	7.1	
12 months	↑PTH	0		6.7	1	20	3.5	0		3.2	2	33.3	3.0
N=21	↓PTH	2	66.7	(2.6-)	4	80	(1.6-4.8)	8	80	(2.1-5.7)	3	50.0	(1.3-6.5)
	Normal PTH	1	33.3		0			2	20		1	16.7	
18 months	↓PTH	1	33.3	5.0	0		7.7	0		5.3	0		2.5
N=16	Normal PTH	1	33.3	(0.9-)	3	50.0	(3.5-15.3)	5	83.3	(1.7-6.9)	2	100	(1.7-)
	↑PTH	1	33.3		3	50.0		1	16.7		0		
24 months	↓PTH	1	33.3	3.7	0		5.5	0	0	5.3	0		3.2
N=12	Normal PTH	2	66.7	(1.1-4.1)	2	100	(3.8-)	3	100	(1.7-6.9)	3	75	(1.9-8.8)
	↑PTH	0			0			0	0		1	25	

PTH reference range; 1.7-7.5 pg/ml

5.4 DISCUSSION

This is the first prospective cohort study investigating plasma 25(OH)D levels at diagnosis and during treatment in paediatric cancer patients from Scotland. These results are novel and importantly show that this cohort had a high prevalence of plasma 25(OH)D inadequacy (deficiency and insufficiency) during the whole study period. Plasma 25(OH)D levels in paediatric cancer patients and age matched healthy controls were similar; however, unlike healthy controls, the paediatric cancer cohort showed no seasonal variation and a higher prevalence of deficiency. Moreover, children diagnosed with solid tumours followed by haematological malignancies exhibited the lowest plasma 25(OH)D levels and the only effective method to achieve optimal plasma 25(OH)D levels was by supplementing with vitamin D. Older age was identified as the only statistically significant factor contributing to plasma 25(OH)D inadequacy at baseline, and overnutrition and non-supplementation during treatment.

5.4.1 Prevalence of plasma 25(OH)D

Contrary to findings from a study in neighbouring North England (Sinha et al. 2011), this study showed that the overall plasma 25(OH)D levels of newly diagnosed paediatric cancer patients and healthy children was comparable. This suggested that paediatric cancer patients from Scotland were not at higher risk of plasma 25(OH)D inadequacy than healthy children at the time of diagnosis. Nonetheless, the prevalence of plasma 25(OH)D deficiency was higher in newly diagnosed paediatric cancer patients than in healthy children (29% v 23%) as opposed to insufficiency, which was higher in healthy children (40% v 35%). However, these levels are lower than those reported in paediatric cancer patients from Europe (Revuelta Iniesta et al. 2015b). Of note, there was a higher representation of winter samples in the healthy controls than the paediatric cancer cohort (30% v 43%), which might have contributed to the unexpectedly higher prevalence of vitamin D inadequacy in the healthy controls.

Current UK guidelines on vitamin D are aimed at the healthy children population and stipulate that children under the age of 5 years should be supplemented with 7.5-

10µg/day (300-400 IU) of vitamin D, and those who are receiving vitamin D fortified foods do not need extra vitamin D (RCPCH 2013). The current study has clearly established that most paediatric cancer patients who were not supplemented were either deficient or insufficient, or eventually became deficient as shown by the high prevalence of plasma 25(OH)D inadequacy (33-50%) throughout the study period. Furthermore, macronutrient supplementation alone prevented plasma 25(OH)D inadequacy but patients rarely reached optimal levels, suggesting that macronutrient supplementation, which is fortified with vitamin D, did not meet the requirements for vitamin D in this population. Finally, vitamin D supplementation taken in the form of multivitamins or as therapeutic supplementation was essential to achieve optimal 25(OH)D levels in all paediatric cancer patients. Remarkably, the present study found that older children were at higher risk of plasma 25(OH)D inadequacy at baseline and therefore would also require supplementation, which is not stipulated in the RCPCH (2013) guidelines. However, it is important to note that three patients on high dose (20.000 IU) vitamin D supplementation became toxic. Therefore, this study recommends vitamin D supplementation for all paediatric cancer patients, but emphasise the need for close monitoring to avoid toxicity.

Unlike healthy children, this paediatric cancer cohort did not show any seasonal variation in plasma 25(OH)D levels, at diagnosis or during treatment. These findings are supported by a study performed in survivors of childhood cancer from the USA (latitude 34ºN) (Rosen et al. 2013), but contrasts with two studies (Modan-Moses et al. 2012, Sinha et al. 2011); one performed in North England (latitude 54.9ºN) during and following cancer therapy (Sinha et al. 2011), and the other performed in Israel (latitude 31ºN) in paediatric cancer patients during therapy (Modan-Moses et al. 2012). Therefore, this study hypothesises that paediatric cancer patients from Scotland are not exposed to enough sunlight during the summer months, probably due to the multiple treatment induce side-effects from the disease and treatment (Sala et al. 2004, Revuelta Iniesta et al. 2015a), and that diet alone is not enough to replenish plasma 25(OH)D stores.

Stratification of the data by type of diagnosis revealed results consistent with a recent systematic review (Revuelta Iniesta et al. 2015b) and a large study (n=2198)

performed in the adult oncology population from USA. Patients diagnosed with solid tumours had prevalence of plasma 25(OH)D inadequacy of 71% and 75% respectively, the current study showed that children diagnosed with solid tumours exhibited the highest prevalence of plasma 25(OH)D inadequacy (69%) at the time of diagnosis. However, it also showed a high prevalence of plasma 25(OH)D inadequacy in children diagnosed with haematological malignancies (65%) at diagnosis, which contrasts with findings from elsewhere (Revuelta Iniesta et al. 2015b, Simmons et al. 2013). Although others have reported similar prevalence of vitamin D inadequacy in children diagnosed with haematological malignancies in Canada (Atkinson et al. 1998, Halton et al. 1996), the measured vitamin D was 1,25-dihydroxyvitamin D, which is not equivalent to plasma 25(OH)D. Children diagnosed with brain tumours and OAD had slightly lower prevalence of plasma 25(OH)D inadequacy at diagnosis and during treatment than other diagnoses; however owing to their small sample size, these results have to be interpreted with caution. Despite some patients having being supplemented with macronutrients and micronutrients, prevalence of 25(OH)D inadequacy remained high throughout the course of treatment for both diagnostic criteria; solid tumours and haematological malignancies. Once more, this highlights the need for a more rigorous approach by health professionals to target patients who are deficient or insufficient and to provide appropriate therapeutic supplementation and monitoring at diagnosis and during the course of treatment.

In line with recent evidence (Sinha et al. 2011, Revuelta Iniesta et al. 2015b), the present study found a relationship between PTH and plasma 25(OH)D in the healthy controls, but not in the paediatric cancer patients. Although, in the healthy population, PTH measured alongside plasma 25(OH)D is considered the most sensitive physiological measure of plasma 25(OH)D status and bone homeostasis (Holick 2009), the present study suggests that there might be other factors influencing their relationship. Unfortunately, this study was unable to investigate these factors due to the relatively small sample; however it has been attributed to the type of cancer and the different forms of treatment, including chemotherapy and corticosteroids, which can lead to nephrotoxicity and hepatotoxicity, in turn

interfering with the 25(OH)D, 1,25(OH)D and PTH metabolism (Atkinson 2008, Zhou et al. 2006). Furthermore, a stronger relationship between plasma PTH and 25(OH)D develops with age (Greer 2009), which might have affected the results from this study, since the healthy controls were slightly older.

5.4.2 Factors contributing to reduced plasma 25(OH)D levels

Consistent with results from a recent meta-analysis (Revuelta Iniesta et al. 2015b), older age was associated with reduced plasma 25(OH)D levels in paediatric cancer patients at baseline. This association was also found in the healthy controls, in line with a study performed in healthy children from the USA (Kumar et al. 2009), which could reflect the widespread issue of vitamin D. Teenagers tend to eat less vitamin D rich foods, especially fortified foods, and spend less time playing outdoors than younger children (Rosen et al. 2013). Alongside infancy, puberty is accompanied by a rapid period of growth and appropriate plasma 25(OH)D levels are essential to allow for optimal growth (Tanner 1990a); thus this population should also be targeted.

Like healthy individuals (Holick 2006), but contrary to other studies investigating factors contributing to plasma 25(OH)D inadequacy in paediatric cancer patients (Rosen et al. 2013, Modan-Moses et al. 2012), the results from the present showed that overnourished children were more likely to have plasma 25(OH)D inadequacy during treatment and this was regardless of nutritional support. An inverse relationship between high BMI and plasma 25(OH)D in the healthy population is well established (Holick 2006), which has been attributed to a reduction in plasma 25(OH)D availability due to the sequestration of vitamin D by adipose tissue (Wortsman et al. 2000).

5.4.3 Limitations of the study and future research

Several limitations related to the methodology of this study have been identified; particularly, the reduced sample size at later stages of the study, which precluded considering factors associated with plasma 25(OH)D at later stages of treatment. Some patients were already on nutritional support at baseline, which could potentially have affected plasma 25(OH)D levels. The higher proportion of winter

samples in the healthy control may have distorted the high plasma 25(OH)D inadequacies reported. The fact that plasma 25(OH)D from the paediatric cancer cohort and the controls was analysed using different techniques and in different laboratories facilities may have added an extra degree of variability between the results obtained. Finally, there were only 2 non-Caucasian patients (dark skin) in both groups, which could explain why lower plasma 25(OH)D levels was not associated with ethnicity. Future research should ideally include large multicentre epidemiological studies that are better able to identify factors contributing to plasma 25(OH)D inadequacy in the different types of cancer during treatment. Also, randomised controls trials in which the effects of vitamin D supplementation on clinical outcome, particularly bone mass density, are warranted.

5.5 CONCLUSION

In conclusion, the current study has highlighted that paediatric cancer patients from Scotland have a high prevalence of plasma 25(OH)D inadequacy at diagnosis and during treatment and that older age, not being supplemented and being overnourished potentially contributes to deficiency and insufficiency. Importantly, this study recommends vitamin D supplementation to all paediatric cancer patients given that macronutrient supplementation alone prevented further vitamin D inadequacy, but rarely produced optimal levels, and high longitudinal inadequacy rates continued throughout the study.

CHAPTER VI

6. ASSESSMENT OF ANTIOXIDANTS, OXIDATIVE STRESS AND POLYUNSATURATED FATTY ACIDS IN PAEDIATRIC CANCER PATIENTS: A PROSPECTIVE COHORT PILOT STUDY

6.1 INTRODUCTION

The findings from chapter IV highlighted that children diagnosed and treated for cancer were at high risk of undernutrition and protein energy malnutrition during the initial phases of treatment; but overnutrition was more prevalent at later stages. Children receiving a high treatment risk protocol were at higher risk of undernutrition also during the initial phases of treatment and there was a high need for nutritional support in this cohort of patients. Moreover, chapter VI showed that paediatric cancer patients from Scotland had a high prevalence of plasma 25(OH)D inadequacy during treatment and that only vitamin D supplementation produced optimal levels.

At present, there is a great deal of interest in the benefits of antioxidants and polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), in health. A protective role against cardiovascular diseases (CVD), neurodegenerative disorders and anti-inflammatory properties has been associated with antioxidant intakes (Ladas et al. 2004, Obrenovich et al. 2011), whilst DHA and EPA have anti-inflammatory properties, which may protect against CVD, cancer and some auto-immune conditions, such as rheumatoid arthritis (Lorente-Cebrián et al. 2013). In clinical practice a nutritional profile consists of assessment of growth, routine biochemical and haematological blood tests only. However, plasma antioxidant status (TAS), antioxidant capacity (TAC) and markers of oxidative stress as well as lipid profile levels may provide a further indicator of nutrient adequacy and functional markers (de la Torre Aguilar et al. 2012, Kuliszkiewicz-Janus et al. 2008, Gleissman et al. 2011, Ladas et al. 2004).

Emerging evidence support a protective role of TAS, TAC and PUFA against chronic conditions. Also, most children required nutritional support during treatment and now survival rates have considerably improved (Wallace et al, 2013). However, children with cancer are at a higher risk of developing the metabolic syndrome, cardiac complications and developing secondary malignancies due to cancer treatments (Wallace et al. 2013). Despite all of this, no study has investigated the profile of TAS, TAC and PUFA and whether nutritional support provides appropriate lipids and antioxidants in this cohort. Therefore, the aims of this study were:

(i) To assess the TAS, TAC and oxidative stress as well as the lipid profile of paediatric cancer patients at diagnosis and 6 months into treatment.

(ii) To establish whether there were any differences in TAS, TAC and oxidative stress as well as the PUFA profile between baseline and 6 months and between the following groups: type of cancer, treatment risk and nutritional support also at baseline and 6 months.

(iii) To establish whether there were any associations between antioxidant intakes and TAS, TAC and oxidative stress and whether this contributes to clinical outcome (event and event free survival).

(iv)To establish whether there were any differences in TAS, TAC and oxidative stress as well as polyunsaturated fatty acids between children who are on nutritional support and those who were not.

6.2 METHODS

6.2.1 Study design, population and time line

A prospective cohort pilot study was performed. The eligibility criteria were: (i) children aged <18 years; (ii) diagnosed with cancer (ICCC-3) (Steliarova-Foucher et al, 2005) or Langerhans Cell Histiocytosis (appendix I); (iii) diagnosed between April-2013 to Jan-2014 (iv) attending the South East Scotland regional centre for Haematology and Oncology at the RHSC, Edinburgh. The authors excluded children who were treated palliatively at the time of diagnosis and stopped monitoring those who became palliative during the study period, out of respect for the patients and their families. Two measurements were taken at different stages of the disease; one in newly diagnosed paediatric cancer patients and the second over a period of 6 months (±3 months) into treatment. All measurements were taken when the patients were followed up for the purpose of the main prospective study, i.e. when all the measurements of growth, body composition, dietary intake and plasma nutritional screening were performed.

6.2.2 Demographics and clinical parameters

Clinical data (diagnosis, treatment protocol, treatment risk (low, medium and high) (Kazak et al. 2012) and length of treatment) and demographic data (age, gender, ethnicity and socio-economic deprivation) were collected from the medical notes. As a proxy marker for socioeconomic deprivation level of individuals, we used individual residence postcodes to assess deprivation level of areas of residence using the Standard Index of Multiple Deprivation (SIMD) (The Scottish Government 2012). SIMD is presented as a quintile where "I" denotes the most deprived and "V" the least deprived. The paediatric cancer cohort was grouped according to the wider definition of solid tumours, haematological cancers, brain tumours and other associated diagnoses. Height (or length) and weight were measured using standard procedures (RRI). BMI centile was calculated and UK BMI growth centiles were used. Nutritional status was classified as underweight (BMI ≤2.3th centile) and overweight (BMI ≥85th) (Cole et al, 1995).

6.2.3 Blood collection, procedure and analysis of samples

Vitamins and minerals of interest to assess TAS were the following: vitamin A, E, zinc, copper and selenium. Blood collection for the analysis of antioxidant status was performed as described in chapter IV, section 4.3.6.

For the assessment of TAC and oxidative stress left over blood from the syringe was placed in a 1.3 ml HEPARIN tube immediately after blood collection. This was then kept in a metal flask filled with ice to keep the sample both cold and protected from the light until this was processed in the laboratory. The length of time the samples remained in the flask varied between 30 minutes and 2 hours. The samples were then processed in the Child Life and Health, University of Edinburgh, Laboratory facilities. To separate plasma from serum the samples were spun at 1.600ppm and at a temperature of 4°C in centrifuge for 15 minutes. The plasma samples were then extracted using a pipette and placed into an Eppendorf tube. These were then stored immediately at -80°C until they were all analysed for antioxidant capacity and oxidative stress at the end of data collection. Antioxidant capacity and oxidative stress were both analysed by Foudil Smail in the QMU Biochemistry laboratory facilities.

- ***Oxygen Radical Absorbance Capacity (ORAC) Antioxidant Assay***

The method used to analyse TAC was the Oxygen Radical Absorbance Capacity (ORAC) Antioxidant Assay (Ou et al. 2001, Girard-Lalancette et al. 2009). ORAC assay is based on the oxidation of fluorescein, which is a fluorescent probe, by peroxyl radicals via a hydrogen atom mechanism activated by 2,2-azobis-2-methyl-propanimidamide (AAPH). This oxidation results in a reduction of the fluorescence intensity (decay), which can be captured over time, with a fluoremeter. The antioxidants present in the blood delay the peroxyl radical oxidation, thus the higher the concentration of antioxidants the longer it will take for the decay to take place. The TAC is quantified by calculating the net area under the fluorescence curve (AUC) generated from the samples. ORAC samples are expressed in µmol of Trolox equivalents (TE) per gram (µmol TE/g) (Ou et al. 2001; Girard-Lalancette et al. 2009). For further methodological details, see appendix VIII

- ***Thiobarbituric Acid Reactive Substances (TBARS) assay***

Oxidative stress was analysed measuring Thiobarbituric Acid Reactive Substances (TBARS) with a TBARS Assay Kit (Cayman, USA). The TBARS method is used for screening and monitoring lipid peroxidation, which is a major indicator of cellular injury and, thus an indicator of oxidative stress (Armstrong et al. 1994). This method is based on quantification of malondialdehyde (MDA), which is a reactive carbonyl compound derived from the effect that unstable polyunsaturated fatty acids have on cells (Armstrong et al. 1994, Yaki 1998). The total lipid peroxidation is quantified in terms of MDA per µmol.

At present the ORAC and TBARS assays are the most widely employed methods of assessing plasma antioxidant capacity and plasma lipid peroxidation respectively, which allowed comparison with published studies. Additionally, both methods were affordable and were fairly specific as the within individual runs coefficient of variation were 0.9-1.7% for ORAC and 1.8-3.3% for TBARS (Ou et al. 2001, Girard-Lalancette et al. 2009, Armstrong and Browne 1994). For full description of both ORAC and TBARS methods, see appendix VIII.

- ***Fatty acid methyl esters (FAME) by gas-liquid chromatography***

50µl of blood left from the syringe was dropped into a Guthrie card, which is a postcard-sized card with specimen collection paper and left to dry. This was then placed in individual foil pockets and stored at -20°C in the Child Life and Health's laboratory, University of Edinburgh. Analyses of DHA, EPA and AA were then carried out in the University of Stirling laboratory facilities by their own research laboratory assistant team. Blood fatty acids profiles were determined by analysis of fatty acid methyl esters (FAME) by gas-liquid chromatography as described by Bell et al. (2011) and expressed as a percentage of the total plasma lipid profile. The analyses of these samples were performed by Prof Gordon Bell and his laboratory team in the Nutrition Analytical Service, Institute of Aquaculture, University of Stirling.

The reference range of lipid peroxidation for both males and females of all ages has been established at 1.86-3.94 µmol and it is expressed in terms of MDA (Richard et al. 1992, Yagi 1998). Additionally, at present there are only DHA, EPA and AA reference ranges specific for Scottish/British adults (Bell et al. 2011), but not for children. However, a recent large cross-sectional study performed in 713 healthy children, aged 8-11 years from Denmark, and attending a school that was taking part in the healthy New Nordic Diet School Meal Study has published baseline plasma lipid levels data (Damsgaard et al. 2014). This Danish study used a similar assay method (Armstrong et al. 2008) than the one used for the current study (Bell et al. 2011), making the plasma lipid results comparable. Thus, for the purpose of this study these references were used: EPA (0.45-0.77), DHA (2.22-3.76) and AA (7.91-10.46) for both girls and boys expressed as a percentage of whole blood total fatty acids (Damsgaard et al. 2014). Finally, no reference ranges exist for antioxidant capacity (ORAC) for either adults or children.

6.2.4 Dietary intake and nutritional support

Vitamin A, vitamin E, zinc, copper and selenium intakes were assessed by using a 24 hour multi-pass recall method (Reilly et al. 2001) and consequently analysed in WinDiets® (Univation Ltd 2005) (Wise, 2005). Information on nutritional treatment was recorded and this was prescribed according to Subjective Global assessment by

the multidisciplinary team and consisted of enteral +/- parenteral nutrition (macronutrient), micronutrient supplementation, and a combination of macronutrients and micronutrients.

6.2.5 Statistical analyses

The Statistical Package for Social Science (IBM-SPSS for Windows Statistics, version 19) was employed to analyse all data. Descriptive statistics were used to evaluate TAS, TAC, oxidative stress (lipid peroxidation) and lipid profiles (DHA, EPA and) at baseline and at 6 months and Wilcoxon-Signed Rank Test was used to establish changes from baseline to 6 months in these variables (aim i). Associations between TAS (vitamin A, vitamin E/Ch, Zinc, Copper and Selenium), TAC (ORAC) and oxidative stress and inflammation (TBARS, ss-CRP) were performed using Spearman's correlation (aim ii); and univariate associations between treatment risk and whether or not the patients were on nutritional support were established by $\chi 2$-test (aims ii and iii). Mann-Whitney test was used to establish whether there were any differences in TAS, TAC and oxidative stress, and also in fatty acids profiles between children who were on nutritional support and those who were not (aim iv). Finally Spearman's correlation were used to established whether there were any associations between antioxidant intakes (vitamin A, vitamin E, Zinc, Copper and Selenium) and antioxidant status, antioxidant capacity and oxidative stress (aim iv). $P < 0.05$ was considered statistically significant. The STROBE guidelines were followed for the presentation of the data (www.strobe-statement.org).

6.2.6 Ethics

This study used the same population as in "The determinants of nutritional risk in paediatric cancer patients". Thus, there were not changes that impacted on the volunteers, but only in the processing and analysis of the blood, no additional ethical approval was needed. All patients' data were anonymised and kept confidential.

6.3 RESULTS

6.3.1 Demographic and clinical characteristics

Plasma samples were collected from 20 paediatric cancer patients to measure TAS, TAC (ORAC), oxidative stress (TBARS) and PUFA (DHA, EPA and AA) at

baseline. Of these, 16 were also collected at six months and the remaining 4 were not available for the following reasons: treatment given outside reach (n=2) and no routine bloods performed at the time of the measurements (n=2). The baseline demographic and clinical characteristics of the population are presented in table 6.1. Twelve different treatment protocols were used to treat this paediatric cancer cohort, the median (IQR) time from the time of diagnosis until baseline measurements was 15.5 (11.0-21.5) days and from baseline to the second measurement at 6 months (+/- 3 months) was 101 (72.2-160) days. BMI centile expressed as median (IQR) increased from [47.5 (18.5-71.2)] at baseline to [54.0 (23.5-73.2)] at 6 months; however this was not significant (p=0.7).

Table 6-1 Demographic and clinical characteristics of the paediatric cancer cohort (n=20) at baseline

Paediatric cancer cohort	**Median**	**IQR**
Age at diagnosis (years)	4.2	1.5-8.5
	n	**%**
Gender		
male	10	50
Female	10	50
Ethnicity		
White	18	90
Non-white	2	10
SIMD		
I	4	20
II	1	5
III	4	20
IV	8	40
V	3	15
Diagnostic criteria		
Solid tumours	6	30
Haematological malignancies	12	60
Brain tumours (low grade Glioma)	1	5
OAD (LCH)	1	5
Treatment Risk		
Low risk	7	35
Medium risk	5	25
High risk	8	40

LCH: Langerham's cell Histiocytosis; SIMD: Standard Index of Multiple Deprivation presented as a quintile where "I" denotes the most deprived and "V" the least deprived.

6.3.2 Plasma antioxidants, oxidative stress and PUFA levels of paediatric cancer patients

Changes from baseline to 6 months in plasma TAS, TAC and PUFA levels are presented in table 6.2. There were no significant changes in any of the antioxidant status parameters measured; vitamin A, vitamin E/Ch, copper, selenium and zinc or TAC (ORAC). However, the latter increased from a median (IQR) of 83.0 (71.5-89.0) at baseline to 90.2 (74.4-97.3) µmol TE/g at 6 months. In contrast, the median (IQR) plasma lipid peroxidation significantly decreased from 7.4 (6.2-9.0) at baseline to 5.3 (4.5-6.4) µmol/MDA at 6 months (p=0.003) and ss-CRP, although non-statistically significant, also decreased from 4.0 (1.0-8.0) at baseline to 1.0 (1.0-4.0) mg/L at 6 months. Finally, the median (IQR) plasma DHA and AA (expressed as a percentage) significantly increased from 1.3 (0.9-1.9) to 1.8 (1.3-2.1) and from 6.0 (5.4-6.8) to 7.3 (5.5-8.1) respectively, whilst no significant changes were seen in either plasma DHA or AA/EPA ratio.

Vitamin A levels were within the normal range in 76.5% (13/17) of paediatric cancer patients at baseline and 94% (15/16) at 6 months, whilst 12% (2/17) had hypovitaminosis A at baseline and 6% (1/16) at 6 months. Twelve percent (2/17) of patients had hypervitaminosis at baseline and none at 6 months. Vitamin E/Ch levels were within the normal range in 94% (16/17) of patients at baseline and in 100% (16/16) at 6 months, whilst 6% (1/16) had hypervitaminosis E/Ch at baseline. The prevalence of high lipid peroxidation was 95% (19/20) at baseline and 94% (15/16) at 6 months (normal range: 1.86-3.94 µmol/MDA) (Yagi 1998, Richard et al. 1992).

Most paediatric cancer patients had plasma PUFA levels below the reference ranges (Damsgaard et al. 2014). The prevalence of paediatric cancer patients with low EPA (<0.45% of total whole blood weight) was 70% (14/20) at baseline and 60% (12/16) at 6 months. DHA was low (<2.22%) in 95% (19/20) at baseline and in 87.5% (14/16) at 6 months, and the ratio AA/EPA was high (>14.59%) in 100% (20/20) of patients at baseline and in 75% (12/16) at 6 months. Interestingly, AA was also low (<7.91%) in 60% (12/20) and 50% (8/16) of patients at baseline and 6 months respectively.

Table 6-2. Plasma antioxidants, oxidative stress and PUFA of paediatric cancer patients at baseline and 6 months

Plasma levels	Baseline		6 months		P value[1]
	Median	IQR	Median	IQR	
Vitamin A (µmol/L)	0.85	0.7-1.3	1.2	0.87-1.42	0.2
Vitamin E/Ch (µmol/L)	6.05	4.6-7.0	5.6	4.9-6.8	0.9
Copper (µmol/L)	16.6	10.9-21.0	16.8	14.1-18.3	1
Selenium (µmol/L)	0.97	0.8-1.4	0.9	0.8-1.1	0.3
Zinc (µmol/L)	10.4	8.9-20.2	10.7	9.1-13.0	0.4
ORAC (µmol TE/g)	83.0	71.5-89.0	90.2	74.4-97.3	0.3
TBARS (MDA µmol)[2]	7.40	6.20-9.00	5.30	4.50-6.40	0.003
CRP mg/L	4.0	1.0-8.0	1.0	1.0-4.0	0.1
EPA (20 5n-3) %	0.4	0.3-0.5	0.4	0.3-0.6	0.6
DHA (22 6n-3) %	1.3	0.9-1.9	1.8	1.3-2.1	0.001
AA (20 4n-6) %	6.0	5.4-6.8	7.3	5.5-8.1	0.05
AA/EPA ratio (%)	17.1	10.5-22.2	18.7	13.3-25.0	0.3

AA: Arachidonic acid; CRP: C-reactive protein; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; ORAC: Oxygen Radical Absorbance Capacity; TBARS: Thiobarbituric Acid Reactive Substances; [1]Wilcoxon-Signed Rank Test; [2]TBARS reference range for human plasma lipid peroxidation level is 1.86 -3.94 uM in terms of MDA.

Stratification of the data by treatment risk (figure 6.1 and figure 6.2) showed that plasma vitamin A levels were highest in the high treatment risk group in comparison with both low and medium treatment risk groups (figure 6.1a), whilst plasma vitamin E/Ch did not differ (figure 6.1b) at either baseline or 6 months. Also, plasma lipid peroxidation levels (TBARS) of paediatric cancer patients on a high treatment risk protocol were higher than those on low and medium treatment risk protocols at both time points (figure 6.1c). In contrast, antioxidant capacity (ORAC) did not differ in any of the treatment risk groups (figure 6.1d).

Interestingly, DHA and EPA levels were highest in children treated with high treatment risk groups followed by medium and low treatment (figures 6.2a and 6.2b). Additionally, AA levels did not differ between any of the groups at baseline and were highest in the low treatment group at 6 months. Finally, AA/EPA ratio was highest in those treated with low treatment risk protocols in comparison with high and medium treatment risk (figure 7.2d).

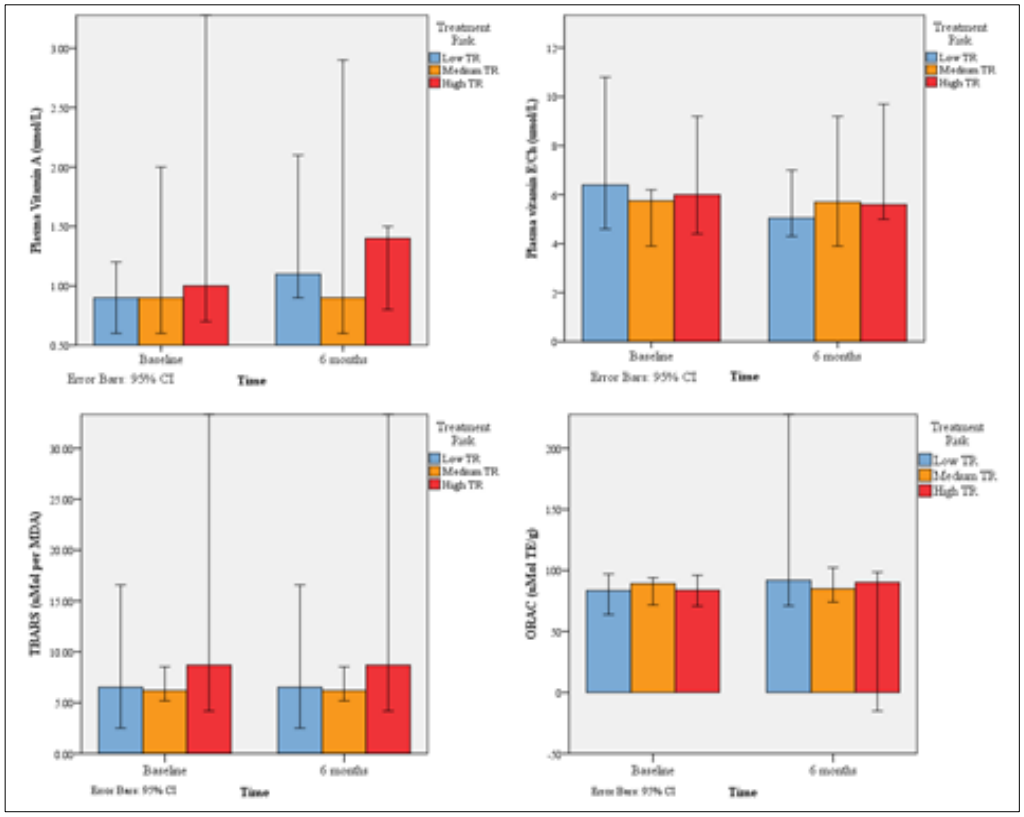

Figure 6.1 Plasma antioxidant levels, antioxidant capacity and oxidative stress in paediatric cancer patients with data stratified by treatment risk.

Figure 6.1a (top left) Plasma vitamin A levels; figure 6.1b (top right) Plasma vitamin E/Ch levels; figure 6.1c (bottom left) Plasma lipid peroxidation levels (TBARS); 6.1d (bottom right) Plasma antioxidant status (ORAC).

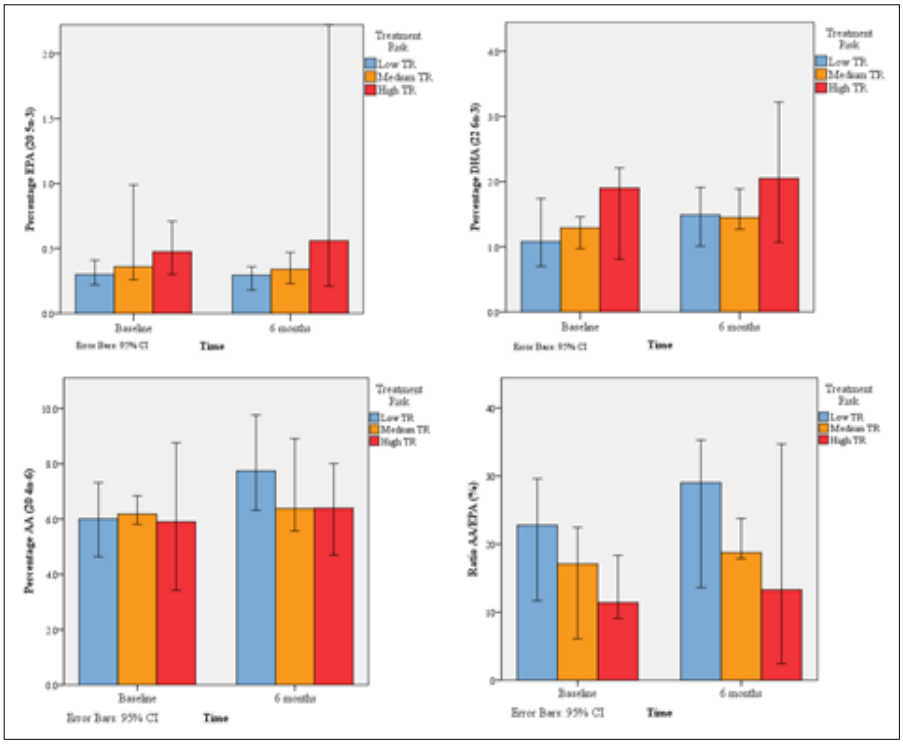

Figure 6.2 Plasma PUFA levels in paediatric cancer patients with data stratified by treatment risk

Figure 6.2a (top left) Plasma eicosahexanoic acid (EPA) expressed as a percentage; figure 6.1b (top right) Plasma decosahexanoic acid (DHA) expressed as a percentage; figure 6.1c (bottom left) Plasma arachidonic acid (AA) expressed as a percentage; 6.1d (bottom right) Plasma AA/EPA ratio expressed as a percentage.

6.3.3 Associations between plasma antioxidant status and capacity, oxidative stress and lipid levels

Spearman's correlations were performed to establish whether there were any associations between the following variables: TAS, TAC and oxidative stress. The antioxidants plasma vitamin A [r=0.04 (very weak); p=0.9 (baseline) and r=0.4 (moderate); p=0.2 (6 months)] and vitamin E/Ch [r=0.1 (weak); p=0.8 (baseline) and r= 0.003 (very weak); p=1 (6 months)] were not significantly associated with antioxidant capacity. Likewise, no significant correlation was found between lipid peroxidation and antioxidant capacity at baseline [r= -0.4 (moderate); p=0.07] or 6 months [r=0.05 (very weak); p=0.8]. However, lipid peroxidation correlated with

copper [r=-0.9 (very strong); p<0.001] and zinc [r= -0.4 (moderate); p=0.01] at baseline.

Further Spearman's correlation analyses were performed to establish whether oxidative stress was associated PUFA levels and BMI centile. Lipid peroxidation (TBARS) did not correlate with any of the following parameters at baseline or 6 months: plasma DHA [r=0.3 (moderate); p=0.2 (baseline) and r=0.1 (weak); p=0.6 (6 months)] and EPA [r=0.3; (moderate); p=0.2 (baseline) and r=0.2 (weak); p=0.5 (6 months)], but it positively correlated with AA levels [r=0.8 (very strong); p<0.001] and negatively correlated with BMI centile at [r= -0.5 (moderate); p=0.04] at baseline. Finally, only DHA positively correlated with BMI centile [r=0.9 (very strong); p<0.001] at baseline.

6.3.4 Dietary antioxidants and nutritional support

The median (IQR) antioxidant intakes of paediatric cancer patients at baseline and 6 months are presented in table 6.3 and Spearman's correlation between dietary antioxidants and corresponding plasma TAS, TAC (ORAC) and oxidative stress (TBARS) are presented in table 6.4.

Table 6-3 Antioxidant intakes of paediatric cancer patients at baseline and 6 months

Antioxidant intakes	**Baseline n=20**		**6 months n=18**		**P value**[1]
	Median	IQR	Median	IQR	
Vitamin A (µd/day)	971	971-1732	264	264-820	<0.001
Vitamin E (mg/day)	8.6	4.0-9.2	14	5.0-13.0	0.7
Copper (mg/day)	0.7	0.5-0.75	1.7	1.7-1.8	<0.001
Selenium (µg/day)	41	15-41	28	21-36.5	0.6
Zinc (mg/day)	6.1	6.1-7.7	8.0	7.5-15.6	<0.001

[1]Wilcoxon-Signed Rank Test

At baseline, there were 8/20 (40%) patients on nutritional support. Of these, all of them (100%) were on macronutrient supplementation; 3/8 (37.5%) were on ONS, 3/8 (37.5%) on NG feeding and 2/8 (25%) on TPN. At 6 months, 12/16 (75%) patients were on nutritional support. Of these 6/12 (50%) were on micronutrient, 4/12 (33%) were on macronutrient supplementation only; of which 3/4 (75%) were on either NG or PEG feeding and 1/4 (25%) on ONS, and 2/5 (40%) were on complex nutritional

support (NG-feed and TPN). Finally, one patient was on both micronutrient and macronutrient supplementation. Three out of 20 (15%) patients were on nutritional support at both baseline and 6 months. Of these, 2/3 patients were on macronutrient supplementation and 1/3 was on both macronutrient (NG-feeding) and micronutrient supplementation. The median (IQR) time the patients were on nutritional support was 11.5 (2.2-17.8) days at baseline and 82.5 (55.7-100) days at 6 months.

Table 6-4 Correlations between dietary antioxidants and plasma antioxidant levels, antioxidant capacity and oxidative stress

Dietary antioxidants	**Corresponding plasma antioxidant**		**Antioxidant capacity (ORAC)**		**Oxidative stress (TBARS)**	
	Baseline	6 months	Baseline	6 months	Baseline	6 months
Vitamin A (µg/day)	r=0.9; p<0.001	r=0.7; p=0.001	r=0.5 p=0.03	r=0.9; p<0.001	r=0.7; p<0.001	r=0.5; p=0.01
Vitamin E (mg/day)	r=0.9; p<0.001	r=0.2; P=0.6	r=-0.3; p=0.2	r=0.7; p=0.001	r=-0.7; p=0.001	r=-0.4 p=0.1
Copper (mg/day)	r=0.7; p=0.01	r=0.5; P=0.02	r=0.7; p=0.008	r=0.5 p=0.03	r=-0.8; p<0.001	r=-0.1; p=0.6
Selenium (µg/day)	r=-0.5; p=0.03	r=0.9; p<0.001	r=-0.3; p=0.1	r=0.8; p<0.001	r=-0.8; p<0.001	r=-0.9; p<0.001
Zinc (mg/day)	r=0.7; p<0.001	r=0.9; p<0.001	r=0.7; p<0.001	r=0.9; p=0.001	r=0.6; p=0.003	r=0.9; p<0.001

R: correlation coefficient; strength of the relationship: 0.0 to ±0.1 (none); ±0.1 to ±0.3 (weak); ±0.3 to ±0.5 (moderate); ±0.5 to ±0.7 (strong); ±0.7 to ±1.0 (very strong) (Bland 2000)

Table 6.5 shows that there were no statistically significant differences between paediatric cancer patients who were on nutritional support and those who were not at either baseline or 6 months in the following parameters measured: TAC (ORAC), TAS (vitamins: vitamin A and vitamin E/Ch; minerals: zinc, selenium and copper) and oxidative stress (TBARS). Likewise, EPA, DHA and AA/EPA ratio (all expressed as percentages) did not statistically differ between these two groups at baseline and 6 months.

Table 6-5 Plasma antioxidants, oxidative stress and PUFA levels of paediatric cancer patients at baseline and 6 months with data stratified by nutritional support.

	Baseline					6 months				
	NS		No NS		P value[1]	NS		No NS		P value[1]
	Median	IQR	Median	IQR		Median	IQR	Median	IQR	
Vitamin A (µmol/L)	1.2	1.0-2.6	0.9	0.7-1.2	0.3	1.0	0.8-1.4	1.4	1.4-1.9	0.2
Vitamin E/Ch (µmol/L)	8.5	4.4-10.0	6.2	5.6-6.4	1	5.8	5.0-6.8	5.5	4.9-5.6	0.1
Copper (µmol/L)	16.8	12.9-23.4	14.5	9.4-18.6	0.4	16.8	13.7-19.5	16.3	14.5-17.7	0.9
Selenium (µmol/L)	0.8	0.6-1.2	12	0.8-1.4	0.2	0.9	0.7-0.9	1.2	0.9-1.5	0.05
Zinc (µmol/L)	16.6	11.1-26.1	9.9	9.1-18.4	0.3	10.6	9.4-12.5	15.6	7.7-25.4	0.2
ORAC (µmol TE/g)	82.6	78.8-85.3	87.2	71.3-91.9	0.4	84.9	75.5-97.7	90.2	81.1-90.9	0.6
TBARS (MDA µmol)[2]	8.5	6.5-11.2	6.2	5.2-8.0	0.2	5.3	4.5-6.4	5.5	4.3-6.4	1
CRP mg/L	1.0	1.0-6.0	3	1.0-9.0	0.5	1.0	1.0-3.5	1.0	1.0-4.0	0.9
EPA (20 5n-3) %	0.4	0.3-0.6	0.4	0.3-0.5	0.9	0.4	0.3-0.6	0.3	0.2-0.5	0.3
DHA (22 6n-3) %	1.4	1.1-1.9	1.3	0.9-1.8	0.8	1.9	1.4-2.2	1.7	1.0-1.9	0.3
AA (20 4n-6) %	5.8	5.3-6.7	6.2	5.2-7.1	0.5	6.9	5.5-7.9	7.5	5.5-8.9	0.6
AA/EPA ratio (%)	12.1	9.4-22.5	17.7	12.1-22.7	0.4	18.3	9.0-22.6	19.8	15.3-33.6	0.2

AA: arachidonic acid; CRP: C-reactive protein; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; ORAC: Oxygen Radical Absorbance Capacity; NS: Nutrition support; TBARS: Thiobarbituric Acid Reactive Substances; [1]Mann-Whitney test.

Interestingly, 6/8 paediatric cancer patients classified as high treatment risk were on nutritional support at baseline in comparison with 1/6 patients classified as low treatment risk and 1/5 as low treatment risk. There was a significant association between high treatment risk and whether the patients were on nutritional support at baseline (Fisher's Exact test, $p<0.02$); however, no significant associations between treatment risk and nutritional support were established at 6 months.

6.4 DISCUSSION

This is the first prospective cohort pilot study investigating plasma TAS, TAC and lipid peroxidation as well as the PUFA's profile of paediatric cancer patients from South East Scotland. The current study has shown that 95% and 94% of paediatric cancer patients had lipid peroxidation levels above the reference range at baseline and 6 months respectively, and that this cohort was most stressed at baseline despite antioxidant status remaining at similar levels. Interestingly, TAC increased slightly following 6 months of treatment. Moreover, there was a high percentage of paediatric cancer patients with low levels of EPA (70% at baseline and 60% at 6 months) and DHA (95% at baseline and 87.5% at 6 months) and, high levels of AA/EPA ratio (100% at baseline and 60% at 6 months). Although, DHA levels improved following 6 months of treatment, AA also increased, whilst EPA remained the same. As a consequence the ratio between AA and EPA also increased.

Children on high treatment risk protocols exhibited the highest lipid peroxidation and plasma vitamin A levels during the study period, whilst antioxidant capacity and the remaining antioxidants measured (Vitamin E/Ch, copper, zinc and selenium) were similar in all treatment risk groups. It was also found that nutritional support did not make any difference to plasma TAS, TAC and oxidative stress (lipid peroxidation) in this cohort; however vitamin E, selenium and copper intakes significantly contributed to higher plasma TAS, higher TAC and lower lipid peroxidation. Finally, as no correlations were found between plasma TAS, TAC and oxidative stress, the current pilot study rejects the hypothesis that the assessment of all vitamins and minerals with antioxidant function are a reliable marker of antioxidant capacity as this appears to be affected by some vitamins and minerals with antioxidant function, but not others and by oxidative stress instead.

6.4.1 Plasma antioxidant levels, antioxidant capacity and oxidative stress

The current study showed that none of the plasma antioxidant levels, which included vitamin A, vitamin E/Ch, zinc, copper and selenium, significantly changed over the 6 months study period. The plasma vitamin A and vitamin E/Ch results are in line with findings from "the determinants of nutritional risk in paediatric cancer" (chapter IV) and have extensively been discussed in regards to other literature in section 4.5.5.1. In line with the current results, a study from Brazil in which 45 children diagnosed with and treated for ALL reported that neither zinc nor copper significantly changed during treatment (Sgarbieri et al. 2006). Likewise, no changes in selenium levels were reported in a French study, in which 170 children diagnosed with and treated for paediatric cancer were included (Malvy et al. 1997). However, unlike the current study, Malvy et al. (1997) reported a significant decrease in zinc levels and Pazirandeh et al. (1999) a reduction in selenium levels in children with ALL receiving treatment. It is well established that zinc, copper and selenium are acute phase reactants and their plasma concentration may change during inflammation (Galloway et al. 2000). Plasma zinc and selenium may decrease during inflammation (Shenkin 2006). The reason for a reduction in plasma zinc levels has been attributed to an increased uptake by the liver to allow zinc to bind to the protein matallothionein, which acts as an antioxidant; whilst a fall in plasma selenium concentration has been attributed to an increase in capillary escape of selenoprotein P, which is the main selenium containing protein in plasma (Shenkin 2006). The discrepancies seen between the current study and those published elsewhere (Malvy et al. 1997, Pazirandeh et al. 1999, Sgarbieri et al. 2006), could therefore be attributed to the timing of measurements as all baseline bloods from the current study were collected while the patients were receiving treatment compared with the other studies, which were collected at diagnosis, before the treatment had started, thus allowing for the changes to occur.

Contrary to all studies published to date, which have investigated antioxidant capacity and oxidative stress using a variety of techniques [antioxidant capacity using ORAC (Kennedy et al. 2005) or different techniques (Battisti et al. 2008, Mazor et al. 2008, Al-Tonbary et al. 2011, Neyestani et al. 2007, Sentürker et al.

1997, Papageorgiou et al. 2005, Protas et al. 2010, Nakagawa 2000), and oxidative stress using TBARS (Battisti et al. 2008, Al-Tonbary et al. 2011) or different techniques (Sentürker et al. 1997, Protas et al. 2010, Stachowicz-Stencel et al. 2011, Caron et al. 2009, Miketova et al. 2005, Stenzel et al. 2010)], the present study found that paediatric cancer patients were more stressed and had lower plasma TAC levels at baseline, soon after the commencement of treatment, than 6 months into treatment. This may reflect that treatment protocols are most intensive during the first 3 months, especially in the ALL group, which represented 60% of the total cohort. Furthermore, children in the high treatment risk group had higher levels of lipid peroxidation, which further confirms that more intensive treatments are associated with higher oxidative stress.

The current study showed that antioxidant intakes contributed to higher plasma TAS and TAC, but helped reduced oxidative stress (lipid peroxidation). Also, lower BMI centiles were associated with higher oxidative stress, which suggests that both undernutrition and reduced antioxidant intake may lead to more cell damage and may explain the higher toxicity rates seen in undernourished children as reported somewhere else (Sala et al. 2012, Green et al. 2008). Unfortunately, due to the small sample size and the short follow up period, the current study was unable to establish any associations between TAC, oxidative stress and clinical outcomes, and the short and long term implications of reduced TAC and high oxidative stress during the treatment phase remain to be elucidated. TAS and TAC have been associated with reduced chemotherapy toxicity, delays in treatment and thus days of hospital stay in children with ALL (Kennedy et al. 2004a, Kennedy et al. 2005) and in children with soft tissue sarcoma and neuroblastoma (Stachowicz-Stencel et al. 2011). However, plasma zinc and copper did not correlate with prognosis in children with ALL (Sgarbieri et al. 2006). As most evidence agreed that higher oxidative stress is associated with poorer clinical outcomes (Al-Tonbary et al. 2011, Caron et al. 2009, Stenzel et al. 2010), it is fair to assume that a higher antioxidant intake would reduce oxidative stress and be in turn beneficial for this cohort.

6.4.2 Plasma PUFA levels

The present pilot study showed that paediatric cancer patients had low levels of EPA and DHA and high levels of AA/EPA ratio. Similar findings have been reported in a small cohort of children diagnosed with a CNS tumour (de la Torre Aguilar et al. 2012) and in adults diagnosed and treated for NHL (Cvetkovic et al. 2010). Moreover, the current study showed that children with higher AA levels also had higher lipid peroxide levels. AA is not only a precursor of inflammatory markers (Lorente-Cebrián et al. 2013), but it is easily oxidised and the main precursor of MDA; therefore contributing further to oxidative stress (Rahal et al. 2014). Interestingly, plasma copper and zinc concentration negatively correlated with lipid peroxidation, and although only plasma copper was statistically significant, this study suggest that copper and zinc may have an essential role at reducing oxidative stress caused by MDA in this cohort of patients during cancer therapy. Copper and zinc are co-factors for the metalloenzyme called superoxide dismutase, which dismutase (deo-oxidise) the extremely toxic MDA into the less toxic hydrogen peroxide (Rahal et al. 2014).

Unlike healthy individuals (Lorente-Cebrián et al. 2013, Greene et al. 2011, Calder 2010, Rahal et al. 2014), this study did not establish that higher levels of whole blood EPA% and DHA% were associated with lower plasma lipid peroxide, but they were negatively associated with ss-CRP. No studies including paediatric cancer patients have investigated this relationship, however such findings have been established in adults with chronic conditions such as cancer (van der Meij et al. 2011, Mocellin et al. 2013) and cardiovascular diseases (Lorente-Cebrián et al. 2013, Bays et al. 2015). In line with the current studies, others have also reported a positive association between PUFA levels, particularly DHA, and nutritional status (Silva et al. 2012, Finocchiaro et al. 2012).

6.4.3 Nutritional support

The TAS, TAC and oxidative stress as well as the PUFA profile (DHA and EPA) of paediatric cancer patients on nutritional support and those who were not did not statistically differ. Furthermore, children on high treatment risk protocols tended to be more nutritionally supplemented than those in low or medium risk treatment

protocols. Given that the current study showed a positive relationship between antioxidant intakes and plasma TAS, it can be hypothesised that current nutritional treatment formulas may not support the needs of paediatric cancer patients, especially those receiving intensive treatments. Due to the lack of studies performed in the same population, comparison of these results is difficult. However a randomised-placebo control trial in which 15 children with active Crohn's disease were treated with exclusively polymeric diet or antioxidant (glutamine) enriched polymeric diet found no differences in plasma antioxidant concentration (Akobeng et al. 2007), which contrasts with the results from the current study.

6.4.4 Limitations of the study and future research

There are several limitations related to the methodology of this study, which may have affected the results. An underestimation of the percentage of whole blood EPA and DHA as well as an overestimation of AA/EPA ratio may have occurred in this study as samples were stored for a period of 3 months to 1 year. A reduction of 9% and 4% in EPA and DHA respectively has been reported following 1 month storage at -20ºC; whilst AA is a more stable PUFA with no reduction of stability during this period of time and also stored at -20ºC (Bell et al. 2011). The reduced sample size precluded stratification of the data by diagnostic criteria and to establish associations between TAS, TAC, oxidative stress and lipid profile, and clinical outcomes; including survival and event. Owing to the nature of this population, the collection of fasted samples was impossible, which could potentially have affected both plasma antioxidant concentrations and total antioxidant capacity. Moreover, the type of nutritional support and the time the patients received it was variable, which once more could have affected the results. Antioxidant intake from children who were not receiving nutritional support was assessed using a 24-hour dietary recall, which may have led to some inaccuracies. Finally, the prevalence of PUFA could have been under or overestimated slightly as the reference range used was obtained from healthy Danish children aged 8-11 years old.

Future research should ideally include: (i) larger population based epidemiological studies in which the TAS, TAC and oxidative stress of paediatric cancer patients are followed up for a longer period of time. Studies should take into consideration

treatment induced side-effects, response to therapy and clinical outcome, such as relapse, death and survival; (ii) well-designed RCT investigating the effectiveness of different forms of supplementation, in which doses of antioxidants are taken are also warranted; (iii) Owing to the paucity of evidence and that at present no specific micronutrient (including those with antioxidant function) and PUFA requirements (including EPA and DHA) have been established for paediatric cancer patients or for critically ill individuals (Department of Health 1991), more research is needed to set optimal doses for this population.

6.5 CONCLUSION

In conclusion, this pilot study has highlighted that paediatric cancer patients from SE Scotland have a high prevalence of oxidative stress, which is highest in children treated with high risk treatment protocols and during the initial phases of treatment. Importantly, nutritional support did not contribute to higher levels of antioxidants and did not appear to meet the needs for EPA and DHA of paediatric cancer patients during the study period. Nonetheless, larger high-quality population based studies and also clinical trials are now justified. This should investigate the effects of nutritional support in the short and long term clinical outcome of paediatric cancer patients and in order to establish optimal formulas, these should include different concentrations of antioxidants and PUFA.

CHAPTER VII

7 SUMMARY, CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

7.1 SUMMARY AND GENERAL CONCLUSIONS

The focus of this PhD has been to investigate changes in nutritional status of children and young people diagnosed with and treated for cancer. The principal aims have focused on the definition and assessment of nutritional status, which has been defined as the body's state in relation to the consumption and utilisation of nutrients (BAPEN 2010). A comprehensive assessment of nutritional status includes evaluating anthropometry, body composition and biochemical parameters, including the assessment of micronutrients status, as well as a clinical and dietary assessment. Thus, these concepts have formed the basis of this project and the themes have been explored throughout.

Given the stated aims of the PhD and in order to put the Scottish paediatric cancer population in context, the second chapter looked at the worldwide prevalence of malnutrition in paediatric cancer patients. It also investigated the effects of both under and overnutrition on clinical outcomes in a published systematic review of the literature. The key findings were as follows:

i. Robust epidemiological data is lacking; rather the heterogeneity of the data reporting made it impossible to accurately determine the prevalence of malnutrition in paediatric cancer patients.
ii. Allied to this, the majority of data concerned both children diagnosed with ALL and the clinical problem of undernutrition during treatment, with little information available about other types of cancer and the problem of overnutrition. Most studies investigating obesity concentrated on survivors of childhood cancer many years after treatment. Looking at childhood cancer as a whole, patients are more likely to be undernourished at diagnosis and during the initial phases of treatment and to then become overnourished at the end of therapy.
iii. When data was stratified by type of cancer, this systematic review found that children diagnosed with solid tumours exhibited a higher prevalence of undernutrition, whilst overnutrition was more common in the haematological malignancies, especially ALL.

iv. The paucity of evidence and the variability of outcomes measured in different studies meant that it was impossible to establish whether nutritional status could be used as a prognostic marker for clinical outcome.

v. It was found that in terms of anthropometry there was a wide variation in the criteria and cut off values used by different research groups and clinicians when trying to assess nutritional status.

vi. Many studies failed to make observations at different stages of the disease and treatment. Thus the principal recommendations of this systematic review are for a consensus to be reached internationally regarding the definitions and measurement of nutritional status and then for high-quality population based longitudinal cohort studies to be undertaken and better address this clinical problem.

Informed by this review of the literature, this project aimed to prospectively identify patterns of change in growth, dietary intake and vitamin and mineral levels in paediatric cancer patients from SE Scotland during their course of illness and treatment. Alongside this, it investigated the need for nutritional support and whether or not clinical and nutritional factors contributed to the development of malnutrition in this population.

The most important findings of this study were that the prevalence of malnutrition varied between different types of cancer, treatment risk and at different stages of the disease highlighting the complexity and diversity of this population. Firstly, a high prevalence of undernutrition was seen during the initial phases of treatment, particularly in children diagnosed with solid tumours; whilst an increase in overweight and obesity rates, particularly in children diagnosed with brain tumours and haematological malignancies, were seen at later stages of the disease and after the completion of treatment. Secondly, high treatment risk was the most important factor contributing to undernutrition (assessed by BMI) during the first three months of treatment, whilst children on low treatment risk protocols were at higher risk of becoming overnourished. However, owing to the limited sample size especially during the later stages of the study, these results should be interpreted with caution. Finally, the preliminary analysis of associations performed in this study found that

undernutrition at diagnosis was associated with an adverse event (relapse, becoming palliative or dying); however this result must be taken with caution due to the small sample siz. Additionally, most children who were undernourished at diagnosis also had more severe disease. It must be highlighted that an extra layer of complexity was identified in that the assessment of nutritional status differed between the different measurements employed.

The current study also showed that younger children were at higher risk of becoming undernourished, whilst older children were at higher risk of becoming overnourished. However, malnutrition did not differ between males and females. Poor linear growth was most common in children diagnosed with haematological malignancies, which has been attributed to the intensive treatments and the increasing use of polytherapy. Although, there were signs of catch up growth at two and a half years post-diagnosis, the sample size was too small to represent an accurate picture of linear growth at later stages.

One of the most striking findings in this study was the progressive muscle wasting witnessed in the initial phases of treatment, which remained until the end of the study. This resulted in a relatively high percentage of patients with PEM and the concurrent gradual increase in fat mass. As a result some paediatric cancer patients became PEM with low fat mass (undernutrition), others PEM with healthy fat mass (well-nourished) and some with high fat mass (overnutrition), however only those with PEM and low fat mass were identified as undernourished by using weight (and BMI) as the only method of assessment. These changes in body composition seen in this paediatric oncology cohort combined with some of the associated effects of cancer and its treatment, which includes oedema, weight of tumour and rapid shifts in fluids, highlights the importance of using arm anthropometry and/or BIA alongside BMI to assess nutritional status in this population. This would greatly help to identify undernutrition, PEM and early signs of obesity and ultimately implement appropriate nutritional and physical activity interventions early on.

Almost 70% of paediatric cancer patients received a form of nutritional support, mainly during the initial phases of treatment. Although a higher proportion of

children diagnosed with brain tumours and OAD received nutritional support, more children diagnosed with solid tumours and haematological malignancies required aggressive nutritional treatments, including advanced nutritional support. This was a reflection of both greater risk of undernutrition in these populations and that they had more severe disease and as a consequence had received more intensive treatment. The current study found that total energy intake significantly contributed to overnutrition, established by BMI, and total energy intake exceeded total energy requirements from nine months onwards. The fact that energy intake exceeded energy requirements in most patients regardless of whether they were receiving nutritional support and that almost 70% patients received some form of nutritional support, highlights the active policies currently in place in the Haematology and Oncology ward from both the RHSC of Edinburgh and Ninewells in Dundee at both preventing and treating undernutrition. This might also explain the rapid increase in BMI, which occurred following an initial decline in those patients who were undernourished. However, this study demonstrated that in some patients the recovery in BMI was due to an increase in FM rather than FFM, thus masking PEM in some patients. Therefore, it appears that current nutritional practices have become very successful at reducing undernutrition and its risk. However, the current approach is less effective at identifying and tackling overnutrition during treatment. The reason for this is multifactorial; (i) understaffing, which may have prevented the team to deal with the high nutritional demands of this populations and (ii) clinical governance, including lack of protocols, systems and specific clinical guidelines for the management of paediatric cancer obesity.

Even though almost 70% of patients received some form of nutritional support, this observational study showed abnormal plasma levels of vitamin A, vitamin B12 and folate in a large proportion of paediatric cancer patients during treatment. The reasons for hypovitaminosis A were attributed to reduced dietary intake and inflammation, whilst hypervitaminosis A was attributed to liver impairment and high vitamin A intakes. No clear factors were identified to contribute to abnormal vitamin B12 and folate deficiency. Although, the optimal plasma levels of paediatric cancer patients is unknown, it appeared that the dietary intake (with or without nutritional

support) of this cohort either exceeded or did not meet their vitamin requirements. Once more, caution should be taken at interpreting the results from the vitamin and mineral intakes of patients who were on diet alone due to unreliability of the 24 hour multiple pass method. Consequently, this study proposed that close monitoring of plasma micronutrient levels should be implemented in clinical practice by the medical and dietetic team.

With the increasing numbers of children cared for in paediatric services, and the often complex medical, psycho-social and nutritional needs of paediatric cancer patients, the cost of managing these populations will rise in parallel, especially with the provision of a more specialised multidisciplinary team. Although, the provision of this cost should be in future agendas, at present shortages in staffing numbers, particularly Dietitians, is a reality and indeed the need for a screening tool should be a priority. This screening tool should be able to identify and predict early on children at risk of short and long term undernutrition and overnutrition, so those who are at risk are targeted by a specialist team.

The second theme of this thesis was to investigate vitamin D levels in paediatric cancer patients at different stages of the disease. Firstly, a systematic review that investigated worldwide prevalence and possible causes of vitamin D inadequacy in this population was performed. The results of this review highlighted once more the absence of a universal approach to measuring and defining 25(OH) D status, the failure to treat different types of cancer as different diseases and the fact that different research groups investigated different variables. Thus the principal recommendation of this paper was the urgent need for high-quality population based longitudinal cohort studies. Nonetheless, this systematic review highlighted the possibility of a high prevalence of vitamin D inadequacy, particularly in European paediatric cancer patients, and a meta-analysis showed that older children may be at higher risk of becoming 25(OH) D deficient or insufficient.

As part of this thesis a prospective case-control study investigating plasma 25(OH) D levels and factors that may contribute to inadequate levels in paediatric cancer patients at different stages of the disease was performed. This study showed that

paediatric cancer patients from Scotland are at high risk of becoming 25(OH) D deficient or insufficient during the course of treatment. In agreement with the systematic review performed as part of this thesis older children were at higher risk of 25(OH) D inadequacy at baseline, whilst being overweight and non-supplemented contributed to 25(OH) D inadequacy during treatment. Children diagnosed with solid tumours followed by haematological malignancies exhibited the lowest plasma 25(OH) D levels and the only effective method to achieve optimal levels was by supplementing with vitamin D. Therefore, this study recommends vitamin D supplementation to all paediatric cancer patients given that macronutrient supplementation alone prevented further vitamin D inadequacy, but rarely produced optimal levels, and high longitudinal inadequacy rates continued throughout the study.

The final chapter of the thesis was the assessment of antioxidant levels, antioxidant capacity and oxidative stress as well as the assessment of polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) at baseline and following 6 months of treatment. This pilot study showed that paediatric cancer patients have high levels of oxidative stress, especially during the initial phases of treatment. Antioxidant capacity increased slightly despite antioxidant status remaining at similar levels. Therefore, this study rejected the hypothesis that the assessment of total antioxidant status was a reliable marker of antioxidant capacity. Instead this appeared to be affected by specific antioxidants, such as vitamin E, selenium and copper and by oxidative stress. Importantly, this pilot study showed that nutritional support did not contribute to higher plasma antioxidant, EPA and DHA levels as these were inadequate in most paediatric cancer patients throughout the study period.

7.2 FUTURE DIRECTIONS

The findings from this PhD have highlighted future areas of research:

- Owing to the small incidence of paediatric cancer, the physical and psychological difficulties that children and their families face and the numerous clinical trials, in which they participate, robust multicentre or even

international high-quality population based prospective cohort studies in which paediatric cancer patients are monitored for a longer period of time are warranted. Ideally they should use universally agreed techniques, reference values and thresholds tailored to different populations. Importantly, different type of cancers should be treated as separate entities and the same approach should be applied to the severity of disease. Finally, the factors contributing to malnutrition considered in this study, and also physical activity, should be investigated.

- Large multi-centre cohort studies are very expensive and often difficult to coordinate. Cheaper ways to perform research and ultimately improve the nutritional status of paediatric cancer patients is by sharing databases between centres. Sharing databases for nutritional research could be achieved by implementing appropriate nutritional practices, monitoring and record keeping first. However, in order to identify optimal nutritional practices, it is fisrt essential to perform nutritional quality improvement projects. For a quality improvement project of nutritional practices, nutritional standards tailored to paediatric oncology patients should be developed by searching the literature, consulting with the specialised multidisciplinary team and auditing the nutritional standards against practices. A series of interventions which focus on staff training and areas that need improvement should follow the audits to achieve optimal nutritional management of this population.

- Future high-quality clinical trials should investigate the effects that different nutritional treatments have on the short and long term nutritional status of paediatric cancer patients. The interventions should focus on nutritional formulas and physical activity strategies. Particular emphasis should be placed on protein, PUFA (EPA and DHA) and antioxidant concentrations. Ideally, the measurement of nutritional status should incorporate measurements of growth, body composition, plasma micronutrient and PUFA status as well as a comprehensive clinical assessment.

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APPENDIX I

- **International Classification of Chiildhood Cancer, 3rd edition (ICCC-3)**
- **Intensity of treatment rating scale: classifying the intensity of paediatric cancer treatment**

International Classification of Childhood Cancer, Third Edition

Site Group	ICD-O-3 Histology (Type)	ICD-O-2/3 Site	Recode
I Leukemias, myeloproliferative diseases, and myelodysplastic diseases			
(a) Lymphoid leukemias	9820, 9823, 9826, 9827, 9831-9837, 9940, 9948	C000-C809	011
(b) Acute myeloid leukemias	9840, 9861, 9866, 9867, 9870-9874, 9891, 9895-9897, 9910, 9920, 9931	C000-C809	012
(c) Chronic myeloproliferative diseases	9863, 9875, 9876, 9950, 9960-9964	C000-C809	013
(d) Myelodysplastic syndrome and other myeloproliferative diseases	9945, 9946, 9975, 9980, 9982-9987, 9989	C000-C809	014
(e) Unspecified and other specified leukemias	9800, 9801, 9805, 9860, 9930	C000-C809	015
II Lymphomas and reticuloendothelial neoplasms			
(a) Hodgkin lymphomas	9650-9655, 9659, 9661-9665, 9667	C000-C809	021
(b) Non-Hodgkin lymphomas (except Burkitt lymphoma)	9591, 9670, 9671, 9673, 9675, 9678-9680, 9684, 9689-9691, 9695, 9698-9702, 9705, 9708, 9709, 9714, 9716-9719, 9727-9729, 9731-9734, 9760-9762, 9764-9769, 9970	C000-C809	022
(c) Burkitt lymphoma	9687	C000-C809	023
(d) Miscellaneous lymphoreticular neoplasms	9740-9742, 9750, 9754-9758	C000-C809	024

(e) Unspecified lymphomas	9590, 9596	C000-C809	025
III CNS and miscellaneous intracranial and intraspinal neoplasms			
(a) Ependymomas and choroid plexus tumor	9383, 9390-9394	C000-C809	031
(b) Astrocytomas	9380	C723	032
	9384, 9400-9411, 9420, 9421-9424, 9440-9442	C000-C809	032
(c) Intracranial and intraspinal embryonal tumors	9470-9474, 9480, 9508	C000-C809	033
	9501-9504	C700-C729	033
(d) Other gliomas	9380	C700-C722, C724-C729, C751, C753	034
	9381, 9382, 9430, 9444, 9450, 9451, 9460	C000-C809	034
(e) Other specified intracranial and intraspinal neoplasms	8270-8281, 8300, 9350-9352, 9360-9362, 9412, 9413, 9492, 9493, 9505-9507, 9530-9539, 9582	C000-C809	035
(f) Unspecified intracranial and intraspinal neoplasms	8000-8005	C700-C729, C751-C753	036
IV Neuroblastoma and other peripheral nervous cell tumors			
(a) Neuroblastoma and ganglioneuroblastoma	9490, 9500	C000-C809	041
(b) Other peripheral nervous cell tumors	8680-8683, 8690-8693, 8700, 9520-9523	C000-C809	042
	9501-9504	C000-C699, C739-C768,	042

		C809	
V Retinoblastoma	9510-9514	C000-C809	050
VI Renal tumors			
(a) Nephroblastoma and other nonepithelial renal tumors	8959, 8960, 8964-8967	C000-C809	061
	8963, 9364	C649	061
(b) Renal carcinomas	8010-8041, 8050-8075, 8082, 8120-8122, 8130-8141, 8143, 8155, 8190-8201, 8210, 8211, 8221-8231, 8240, 8241, 8244-8246, 8260-8263, 8290, 8310, 8320, 8323, 8401, 8430, 8440, 8480-8490, 8504, 8510, 8550, 8560-8576	C649	062
	8311, 8312, 8316-8319, 8361	C000-C809	062
(c) Unspecified malignant renal tumors	8000-8005	C649	063
VII Hepatic tumors			
(a) Hepatoblastoma	8970	C000-C809	071
(b) Hepatic carcinomas	8010-8041, 8050-8075, 8082, 8120-8122, 8140, 8141, 8143, 8155, 8190-8201, 8210, 8211, 8230, 8231, 8240, 8241, 8244-8246, 8260-8264, 8310, 8320, 8323, 8401, 8430, 8440, 8480-8490, 8504, 8510, 8550, 8560-8576	C220, C221	072
	8160-8180	C000-C809	072
(c) Unspecified malignant hepatic tumors	8000-8005	C220, C221	073
VIII Malignant bone tumors			
(a) Osteosarcomas	9180-9187, 9191-9195, 9200	C400-C419,	081

		C760-C768, C809	
(b) Chondrosarcomas	9210, 9220, 9240	C400-C419, C760-C768, C809	082
	9221, 9230, 9241-9243	C000-C809	082
(c) Ewing tumor and related sarcomas of bone	9260	C400-C419, C760-C768, C809	083
	9363-9365	C400-C419	083
(d) Other specified malignant bone tumors	8810, 8811, 8823, 8830	C400-C419	084
	8812, 9250, 9261, 9262, 9270-9275, 9280-9282, 9290, 9300-9302, 9310-9312, 9320-9322, 9330, 9340-9342, 9370-9372	C000-C809	084
(e) Unspecified malignant bone tumors	8000-8005, 8800, 8801, 8803-8805	C400-C419	085
IX Soft tissue and other extraosseous sarcomas			
(a)Rhabdomyosarcomas	8900-8905, 8910, 8912, 8920, 8991	C000-C809	091
(b) Fibrosarcomas, peripheral nerve sheath tumors, and other fibrous neoplasms	8810, 8811, 8813-8815, 8821, 8823, 8834-8835	C000-C399, C440-C768, C809	092
	8820, 8822, 8824-8827, 9150, 9160, 9491, 9540-9571, 9580	C000-C809	092
(c) Kaposi sarcoma	9140	C000-C809	093
(d) Other specified soft tissue sarcomas	8587, 8710-8713, 8806, 8831-8833, 8836, 8840-8842, 8850-8858, 8860-	C000-C809	094

	8862, 8870, 8880, 8881, 8890-8898, 8921, 8982, 8990, 9040-9044, 9120-9125, 9130-9133, 9135, 9136, 9141, 9142, 9161, 9170-9175, 9231, 9251, 9252, 9373, 9581		
	8830	C000-C399, C440-C768, C809	094
	8963	C000-C639, C659-C699, C739-C768, C809	094
	9180, 9210, 9220, 9240	C490-C499	094
	9260	C000-C399, C470-C759	094
	9364	C000-C399, C470-C639, C659-C699, C739-C768, C809	094
	9365	C000-C399, C470-C639, C659-C768, C809	094
(e) Unspecified soft tissue sarcomas	8800-8805	C000-C399, C440-C768,	095

		C809	
X Germ cell tumors, trophoblastic tumors, and neoplasms of gonads			
(a) Intracranial and intraspinal germ cell tumors	9060-9065, 9070-9072, 9080-9085, 9100, 9101	C700-C729, C751-C753	101
(b) Malignant extracranial and extragonadal germ cell tumors	9060-9065, 9070-9072, 9080-9085, 9100-9105	C000-C559, C570-C619, C630-C699, C739-C750, C754-C768, C809	102
(c) Malignant gonadal germ cell tumors	9060-9065, 9070-9073, 9080-9085, 9090, 9091, 9100, 9101	C569, C620-C629	103
(d) Gonadal carcinomas	8010-8041, 8050-8075, 8082, 8120-8122, 8130-8141, 8143, 8190-8201, 8210, 8211, 8221-8241, 8244-8246, 8260-8263, 8290, 8310, 8313, 8320, 8323, 8380-8384, 8430, 8440, 8480-8490, 8504, 8510, 8550, 8560-8573, 9000, 9014, 9015	C569, C620-C629	104
	8441-8444, 8450, 8451, 8460-8473	C000-C809	104
(e) Other and unspecified malignant gonadal tumors	8590-8671	C000-C809	105
	8000-8005	C569, C620-C629	105
XI Other malignant epithelial neoplasms and malignant			

melanomas			
(a) Adrenocortical carcinomas	8370-8375	C000-C809	111
(b) Thyroid carcinomas	8010-8041, 8050-8075, 8082, 8120-8122, 8130-8141, 8190, 8200, 8201, 8211, 8230, 8231, 8244-8246, 8260-8263, 8290, 8310, 8320, 8323, 8430, 8440, 8480, 8481, 8510, 8560-8573	C739	112
	8330-8337, 8340-8347, 8350	C000-C809	112
(c) Nasopharyngeal carcinomas	8010-8041, 8050-8075, 8082, 8083, 8120-8122, 8130-8141, 8190, 8200, 8201, 8211, 8230, 8231, 8244-8246, 8260-8263, 8290, 8310, 8320, 8323, 8430, 8440, 8480, 8481, 8500-8576	C110-C119	113
(d) Malignant melanomas	8720-8780, 8790	C000-C809	114
(e) Skin carcinomas	8010-8041, 8050-8075, 8078, 8082, 8090-8110, 8140, 8143, 8147, 8190, 8200, 8240, 8246, 8247, 8260, 8310, 8320, 8323, 8390-8420, 8430, 8480, 8542, 8560, 8570-8573, 8940, 8941	C440-C449	115
(f) Other and unspecified carcinomas	8010-8084, 8120-8157, 8190-8264, 8290, 8310, 8313-8315, 8320-8325, 8360, 8380-8384, 8430-8440, 8452-8454, 8480-8586, 8588-8589, 8940, 8941, 8983, 9000, 9010-9016, 9020,	C000-C109, C129-C218, C239-C399, C480-C488, C500-C559,	116

	9030	C570-C619, C630-C639, C659-C729, C750-C768, C809	
XII Other and unspecified malignant neoplasms			
(a) Other specified malignant tumors	8930-8936, 8950, 8951, 8971-8981, 9050-9055, 9110	C000-C809	121
	9363	C000-C399, C470-C759	121
(b) Other unspecified malignant tumors	8000-8005	C000-C218, C239-C399, C420-C559, C570-C619, C630-C639, C659-C699, C739-C750, C754-C809	122
Not Classified by ICCC or in situ			999

Steliarova-Foucher E, Stiller C, Lacour B, Kaatsch P. International Classification of Childhood Cancer, Third Edition. *Cancer* 2005;103:1457-67.

Intensity of treatment rating scale: classifying the intensity of paediatric cancer treatment

 **Intensity of Treatment Rating (ITR-3)** 

Directions: Please review carefully the criteria at the bottom of the page that lists examples of diseases and treatment modalities under each of the four levels of intensity. Based on the information regarding each patient's disease and treatment, use the criteria at the bottom of this page to circle one number to indicate the intensity of treatment (1, 2, 3, 4). Make your ratings based on the specified criteria, rather than your own clinical judgment.

ID #	ABSTRACTION INFORMATION						INTENSITY RATING
	Diagnosis, including if relapsed	Stage or Risk Level	Treatment Modalities				
			Surgery?	Chemo?	Radiation ?	Transplant ?	
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4
			y n	y n	y n	y n	1 2 3 4

II. Level 1: Least Intensive Treatments

Includes the least intensive treatments, for these diseases or treatment modalities:

- Surgery Only – All tumor types except brain tumors
- Retinoblastoma - Enucleation (unilateral disease) without chemotherapy
- Wilms' Tumor (Stages 1, 2)
- Chronic Myeloid Leukemia - any chemotherapy, including tyrosine kinase inhibitors
- LCH, surgery or steroid injection only

IV. Level 2: Moderately Intensive Treatments

Includes moderately intensive treatments, for these diseases
or treatment modalities:

- Acute Lymphoblastic Leukemia (Low, Standard, or Intermediate Risk; precursor B cell)
- Brain Tumor - One treatment modality, not including biopsy
- Germ Cell Tumors - With chemotherapy or radiation
- Hepatoblastoma - With chemotherapy and surgical resection, no metastatic disease
- Hodgkin Lymphoma (Low/Intermediate risk: all stages except IIIB, IVB)
- Langerhans Cell Histiocytosis (LCH) with chemotherapy
- Neuroblastoma/Ganglioneuroblastoma (Stages 1, 2 w/chemotherapy and Stage 4S)
- Non-Hodgkin Lymphoma (Stages 1, 2, 3 and Groups A, B)
- Retinoblastoma - With chemotherapy
- Rhabdomyosarcoma (Stages 1, 2)
- Thyroid cancer
- Tumor, other – either chemo or radiation alone

III. Level 3: Very Intensive Treatments

Includes very intensive treatments, for these diseases
or treatment modalities:

- Relapse Protocols for Hodgkins & Wilms' Tumor (first relapse) Only
- Acute Lymphoblastic Leukemia (ALL) (High Risk, Very High Risk, T-cell)
- Acute Myeloid Leukemia and Down Syndrome
- Acute Promyelocytic Leukemia (APL)
- Biphenotypic leukemia – treated like ALL
- Brain Tumor - Two or more treatment modalities
- Carcinoma NOS – Two or more treatment modalities
- Ewings Sarcoma
- Hepatoblastoma- With metastatic disease
- Hemophagocytic lymphohistiocytosis (HLH), chemo alone
- Hodgkin Lymphoma (Stages 3B or 4/High Risk)
- Juvenile Mylomonocytic Leukemia (JMML) – Pretransplant/chemo only
- Nasopharyngeal Carcinoma
- Neuroblastoma/Ganglioneuroblastoma (Stages 3, 4) - Without transplant
- Non-Hodgkin Lymphomas (Group C or Stage 4)
- Osteosarcoma
- Rhabdomyosarcoma (Stages 3, 4)
- Soft Tissue Sarcoma – Two or more treatment modalities
- Wilms' Tumor (Stages 3, 4) – Three treatment modalities
- Tumor, other – 2 or 3 treatment modalities

V. Level 4: Most Intensive Treatments

Includes the most intensive treatments, for these diseases
or treatment modalities:

- Relapsed Disease - Excluding Hodgkin Lymphoma, first relapse of Wilms' Tumor, LCH with systemic treatment, or CML with a different tyrosine kinase inhibitor
- Hematopoietic Stem Cell Transplant (HSCT) - All diseases
- Acute Myeloid Leukemia (AML)
- Biphenotypic leukemia – treated like AML
- Juvenile Myleomonocytic Leukemia (JMML) - With transplant
- Brain tumor – with HSCT

*****In case of more than one tumor, rate the tumor that falls into the highest level.**

APPENDIX II

- **Child Invitation to participate in the study**
- **Consent form**
- **Moderators email**
- **QMU ethical approval**
- **Volunteer information sheet**

Queen Margaret University
EDINBURGH

CHILD INVITATION TO PARTICIPATE

"Assessment of reliability, validity and precision of anthropometrical measurements performed in healthy children"

Invitation to take part in the study

- We would like to ask you for help with our study.
- We want to make sure that you understand what is all about before you decide if you would like to take part
- Please talk it over with your mum/dad and take time to decide

What is the study about?

We would like to measure your **weight, height and the thickness of your arm** to help us prepare for a larger project in which children with cancer are involved.

I would recommend wearing **short sleeves** on the day to facilitate taking the measurements.

The purpose of this is to help us get our techniques right to obtain better results with our measurements.

Will it hurt me?

Taking part

Will taking part help me?

Taking part might not help you but you will help the researchers, Ilenia and Raquel, to improve their techniques.

It will also help many children with cancer.

Will anybody know about my measurements

Only the researchers involved in the study as we will not tell anyone else about your measurements

Do I have to take part?

No, this is up to you!

If you don't take part don't worry, that is fine

If you take part but change your mind later on. That is also fine. Just let us know

If you have questions you can speak with:

Raquel Revuelta Iniesta rrevueltainiesta@qmu.ac.uk

Ilenia Paciarotti ipaciarotti@qmu.ac.uk

Dr Jane McKenzie jmckenzie@qmu.ac.uk

Independent advisor (Dr Iain Gow) igow@qmu.ac.uk

Queen Margaret University
EDINBURGH

CONSENT FORM

"Assessment of reliability, validity and precision of anthropometrical measurements performed in healthy children"

I confirm that I have read and understood the information sheet for the above study, that I have had the chance to ask questions, and that I have received satisfactory answers to the asked questions. ☐

I understand that my/my child's participation is voluntary and that I am free to withdraw myself/my child at any time, without giving any reason ☐

I agree to have my child take part in the above study ☐

Name of parent/carer Date Signature

Name of child/adolescent (If considered appropriate) Date Signature

Name of researcher Date Signature

To all parents/carers who have healthy children under the age of 18:

Do you want to know your children's nutritional status?

We are two PhD's students; Raquel Revuelta Iniesta and Ilenia Paciarotti, both from Queen Margaret University, under the supervision of Dr. Jane McKenzie currently undertaking a project in the Royal Hospital of Sick Children and we need your help. We are investigating how cancer and its treatment affect nutritional status in this population group and for that we need to optimise our skills on how to assess this.

The measurements that we will take are the following:

- Weight and height
- Middle upper arm circumference
- Triceps skinfold

We are looking for healthy children and young adults aged between 2 and 18.

If you would like to find out more, please contact:

If you or your child wish to participate or would like more information, please contact:

Raquel Revuelta Iniesta: rrevueltainiesta@qmu.ac.uk

Ilenia Paciarotti: ipaciarotti@qmu.ac.uk

This project has ethical approval.

Raquel Revuelta Iniesta
PhD student
Dietetics, Nutrition and Biological Sciences
Queen Margaret University

Sheila Adamson
Collaborations Development Co-ordinator
Queen Margaret University, Edinburgh
Queen Margaret University Drive
Musselburgh
East Lothian EH21 6UU

Direct Dial
Tel (0)131 474 0000 Fax (0)131 474 0001
Email: sadamson@qmu.ac.uk

07 June 2012

Dear Raquel

Request for Ethical Approval for a Research Project – Assessment of reliability, validity and precision between intra and inter observer anthropometrical measurements performed in children and young adults.

Thank you for your response to the letter I sent you following consideration of your application by the Research Ethics Panel.

Professor Nigel Gleeson, Vice-Convener of the Panel, has reviewed your response to the points you were required to address, and has confirmed that he is happy to take Convener's Action to grant full ethical approval for your research.

A standard condition of this ethical approval is that you are required to notify the Panel, in advance, of any significant proposed deviation from the original protocol. Reports to the University are also required once the research is underway if there are any unexpected results or events that raise questions about the safety of the research. Please find the appropriate form for this enclosed.

We would like to thank you for your co-operation and wish you well with your project.

Yours sincerely,

Sheila Adamson
Collaborations Development Co-ordinator

Queen Margaret University
EDINBURGH

INFORMATION SHEET FOR PARENTS

"Assessment of reliability, validity and precision of anthropometrical measurements performed in healthy children"

This study will be carried out by Ilenia Paciarotti and Raquel Revuelta Iniesta; we are both PhD students at Queen Margaret University under the supervision of Dr. Jane Mckenzie. The purpose of this project is to assess measurements of body composition in healthy children in preparation for an ongoing project at the Royal Hospital of Sick Children (RHSC), which aims to assess nutritional risk in children with cancer.

We are looking for boys and girls aged between 2 and 18.

- This study will involve:
    - Meeting with the children in a private location: QMU clinic room or other suitable place.
    - The following anthropometrical measurements will be taken on your child twice by each of the researchers:
        - Weight – measured clothed, without shoes, on standing scale
        - Height – measured clothed, without shoes, using a standing height measure
        - Middle upper arm circumference - this involves finding the middle point between the elbow and shoulder and measuring the circumference. Measurements are taken on the non-dominant arm

        - Triceps skinfold- this involves measuring the thickness of the skin on the triceps. For this measurement we use paediatric calipers which pinch the skin gently to measure the thickness
    - Wearing short sleeves will facilitate measurement of the arm.
- Will it hurt my child?
    - Your child should not experience any pain, however the triceps skin fold is sometimes perceived as unpleasant by the children.
- Benefits of taking part
    - This study will not benefit your child directly; however the information obtained will help the researchers to conduct the study performed at the RHSC
- Confidentiality and anonymity
    - All children who take part in the project will be given an identification number and all data will be anonymous.
- Withdrawal
    - You or your child will be able to withdraw from the study at any time without providing any explanation.

- Contact details
    - Researchers' name:
        - Raquel Revuelta Iniesta rrevueltainiesta@qmu.ac.uk
        - Ilenia Paciarotti ipaciarotti@qmu.ac.uk
    - Supervisor:
        - Dr. Jane McKenzie jmckenzie@qmu.ac.uk
    - Independent advisor:
        - If you wish to consult a person who is not involved in this research for independent advice, please contact:
        - Dr. Iain Gow igow@qmu.ac.uk

APPENDIX III

- **Child invitation to participate in the study**
- **Consent forms**
- **Parents invitation to participate in the study**

CHILD INVITATION TO PARTICIPATE

"Nutritional Risk in Childhood Cancer (prospective study)"

Invitation to take part in the study

- We would like to ask you for help with our study.
- We want to make sure that you understand what is all about before you decide if you would like to take part
- Please talk it over with your mum/dad and take time to decide what you want to do

What is the study about?

- Becoming underweight can happen if you have cancer.
- Also some kids and teenagers get overweight later
- So, we want to find out more about these problems

What will happen if I agree to take part?

- We will measure your weight and height as usual and also your thickness of your arm.
- We will ask you some questions about your food intake
- We will measure your blood vitamins and minerals
- We will take these measurements when you come for appointments only.
- Finally we will ask you and your mum/dad to tell us about what cancer does to your life by means of some questions on a form called questionnaire

Will it hurt me?

Don't worry!

Taking part will not hurt you

Will taking part help me?

Taking part might not help you but should help other children and teenagers in the future.

Who will know I have taken part?

Only the researchers involved in the study and the doctors if necessary, otherwise nobody else

Do I have to take part?

No, this is up to you!

If you don't take part don't worry, that is fine

If you take part but change your mind later on that is also fine. Just let us know

If you have questions you can speak with:

Raquel Revuelta Iniesta (Research Dietitian) (Tel: 0131 536 0824)

Dr Mark Brougham (Consultant Oncologist)

Kerry White (Staff Nurse)

Prof. David Wilson (Consultant Gastroenterologist) (Tel: 0131 536 0615)

Child Life and Health
Reproductive & Developmental Sciences
Clinical Sciences and Community Health
20 Sylvan
Place EDINBURGH
EH9 1UW
Telephone: 0131 536 0801

Study Number:

Patient Identification Number for this trial:

CONSENT FORM – child 8 to 12 years

Title of Project:

"Nutritional Risk in Childhood Cancer (prospective study)".

Name of Principal Researcher: Dr David C Wilson

Please initial box

I have read or my family have read out to me the study information sheet, ☐

I have had the chance to ask questions and ☐

I am happy with answers to the questions that I have asked. ☐

I understand that my taking part is up to me and that I am can stop at any time, and the doctors will go on treating me in the way they think is best for me ☐

I agree to take part in the above study. ☐

_____ _____ _____

Name Date Signature

_____ _____ _____

Name of Person providing information Date Signature (if different from researcher)

_____ _____ _____

Researcher Date Signature

(Investigator or delegated medically qualified co-investigator)

1 for child, 1 for researcher; 1 to be kept with hospital notes

Child Life and Health
Reproductive & Developmental Sciences
Clinical Sciences and Community Health
20 Sylvan
Place EDINBURGH
EH9 1UW
Telephone: 0131 536 0801

Study Number:

Patient Identification Number for this trial:

CONSENT FORM – child less than 8 years

Title of Project:

"Nutritional Risk in Childhood Cancer (prospective study)".

Name of Principal Researcher: Dr David C Wilson

I have read or my family have read out to me the study information sheet

I have had the chance to ask questions and

I am happy with answers to the questions that I have asked.

I know that I don't have to help unless I want to. If I start, I can stop at any time. The doctors will still try hard to make me better.

I agree to take part in the above study.

_____ _____ _____

Name Date Signature

_____ _____ _____

Name of Person providing information Date Signature (if different from researcher)

_____ _____ _____

Researcher Date Signature

(Investigator or delegated medically qualified co-investigator)

1 for child, 1 for researcher; 1 to be kept with hospital notes

Child Life and Health
Reproductive & Developmental Sciences
Clinical Sciences and Community Health
20 Sylvan
Place EDINBURGH
EH9 1UW
Telephone: 0131 536 0801

Study Number:

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: "Nutritional Risk in Childhood Cancer (prospective study)".

Name of Principal Researcher: Dr David C Wilson

I confirm that I have read and understand the information sheet for the above study, that I have had the opportunity to ask questions, and that I have received satisfactory answers to the questions that I have asked	☐
I understand that my child's participation is voluntary and that I am free to withdraw my child at any time, without giving any reason, without my infant's medical care or legal rights being affected.	☐
I agree to have my infant take part in the above study.	☐
If asked I agree to take part in audio-taped interviews.	☐

_____ _____ _____

Name of Parent/Carer Date Signature

_____ _____ _____

Name of Person providing information Date Signature

(if different from researcher)

_____ _____ _____

Researcher Date Signature

(Investigator or delegated medically qualified co-investigator)

1 for parent/carer, 1 for researcher; 1 to be kept with hospital notes

Child Life and Health

Reproductive & Development Sciences

Clinical Sciences & Community Health

20 Sylvan Place

Edinburgh

EH9 1UW

PARENT INVITATION TO PARTICIPATE

"Nutritional Risk in Childhood Cancer (prospective study)"

This resaerch project will be carried out by the Royal Hospital for Sick Children, Edinburgh, The University of Edinburgh & Queen Margaret University.

The study has the full support and permission of the paediatric haematology and oncology department in Sick Kid's, the hospital and both Universities.

What is the study about?

Malnutrition (undernutrition and overnutrition) is a major concern in the treatment of children and young people with cancer. Undernutrition, loss of weight or failuring to gain weight normally, may occur during the treatment of cancer and also during remission, while obesity is becoming a major concern for the survivors of childhood cancer. Thus children and teenagers who face these issues may need additional help with nutrition. We realise that these nutritional problems are a source of great stress for families as we all wish to provide adequate nutrition to our children.

Why do we need to do this research?

The improvement in medical treatment is achieved by carrying out clinical research and thanks to them we have recently made huge progresses in the treatment and cure of cancer. In order to advance further and to improve health services for children and adolescents with cancer we need to continue doing research.
If being undernoushed and overnourished is as common as we think, then we would be able to argue for greater provision of services (e.g. Dietitians) and care, which will in turn help children and families in the future.

How will we do this?

We will ask the parents of children who have been diagnosed with cancer and are followed up in SE Scotland if they are willing to take part in the research study.

What is involved for my child?

Your child will not receive any extra tests, procedures or interviews, except we will do more frequent measurements of height, weight and some extra growth measurements in the ward and at follow up.

We will ask you to tell us about your child's food intake.

When your child needs to have blood taken for medical reasons we would need a little extra to analyse your child's blood vitamin and mineral levels.

We also wish to measure your child's quality of life and stress levels (stress levels only if your child is 8 years or older) by means of a questionnaire.

What is involved for me?

We wish to measure your thoughts on your child's quality of life by means of the same quality of life (PedsQL) questionnaire. We also wish to measure the effects that your child's cancer has caused you, your stress levels as well as your emotional and physical health by means of a second questionnaire (SF36). Although the questionnaires should only take about 5-10 minutes to fill in you may find that they are time consuming or tiresome.

Where do I have to go?

All the measurements will be taken at your child's follow up visits or during admission to the Royal Hospital for Sick Children (RHSC).

If you or your child want to opt in the study it is entirely up to you. Your child's care will not be affected by whether or not you take part.
If you/your child agree to take part but want to opt out later that is also fine and it will not affect your child's care.

If you have any questions or would like a more detailed explanation of the study, please contact:

Prof. David Wilson (Consultant Gastroenterologist at the RHSC) Tel: 0131 536 0615
Dr Mark Brougham (Consultant Oncologist at the RHSC) Ward 2
Raquel Revuelta Iniesta (Research Dietitian at the RHSC) Ward 2 Tel: 0131 536 0824
Kerry White (staff nurse) ward 2

APPENDIX IV

- **CRP protocol**
- **Ferritin protocol**
- **Folate protocol**
- **Vitamin B12 protocol**

SYMBOLS IN PRODUCT LABELING

CAL	Calibrator
CONC	Concentration
CONT	Contents of kit
CONTAINS: AZIDE	Contains sodium azide. Contact with acids liberates very toxic gas.
CONTROL	Control
FOR USE WITH	Identifies products to be used together
INGRED	Ingredients
IVD	In vitro diagnostic medical device
LOT	Batch code/Lot number
R1	Reagent 1
R2	Reagent 2
REF	Catalog number/List number
SN	Serial number
	Consult instructions for use
	Use by/Expiration date
	Manufacturer
	Temperature limitation

CRP VARIO
REF 6K26-30 and 6K26-41

For product questions contact Abbott Laboratories Customer Support
United States: 1-877-4ABBOTT
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CRP VARIO

REF 6K26-30 and 6K26-41

FOR USE WITH
ARCHITECT

NOTE: This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NOTE: Changes Highlighted

INTENDED USE

The MULTIGENT CRP Vario assay [CRPVa] is intended for the quantitative immunoturbidimetric determination of C-reactive protein in human serum and plasma with variable assay ranges [CRP16, CRP32, CRP48] using the ARCHITECT *c*Systems.

SUMMARY AND EXPLANATION OF TEST

C-reactive protein (CRP) is an acute phase protein whose concentration rises non-specifically in response to inflammation. CRP is seen to increase as a result of the inflammatory process, most notably in response to pneumococcal (bacterial) infection, histolytic disease, and a variety of other disease states. Intraindividual variation is a major limitation of the assay when the assay is used for directing therapies. Intraindividual variations of the CRP levels are from 30% to 60%. Serial measurement may be required to estimate true mean of CRP depending on the intended use in any specific individual. CRP is used as a marker or general diagnostic indicator of infections and inflammation, in addition to serving as a monitor of patient response to pharmacological therapy and surgery.

PRINCIPLES OF PROCEDURE

MULTIGENT CRP Vario is a latex immunoassay developed to accurately and reproducibly measure blood CRP levels in serum and plasma. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change (572 nm), with the rate of change being proportional to the quantity of CRP in the sample. Three different methods (High Sensitivity [CRP16], Standard [CRP32], and Wide Range [CRP48]) are available to cover a wide analytical measurement range.

Methodology: Turbidimetric/Immunoturbidimetric

REAGENTS

Reagent Kit

REF 6K26-30 MULTIGENT CRP Vario is supplied as a two reagent kit, which contains:

R1	2 x 37 mL
R2	2 x 37 mL

REF 6K26-41 MULTIGENT CRP Vario is supplied as a two reagent kit, which contains:

R1	3 x 86 mL
R2	3 x 86 mL

Method	Estimated Tests per Kit*	
	R1 6K26-30	REF 6K26-41
High sensitivity	600	2,192
Standard	600	2,192
Wide range	600	1,836

*Calculation is based on the minimum reagent fill volume per kit.

Reactive Ingredients		Concentration
R1	Glycine buffer (pH 7.0)	1.28%
R2	Anti-CRP polyclonal antibodies (rabbit) adsorbed on latex particles	0.2%

Nonreactive Ingredients:
R1 contains bovine albumin (≤ 1%) and sodium azide (< 0.1%).
R2 contains bovine albumin (≤ 0.1%) and sodium azide (< 0.1%).

REAGENT HANDLING AND STORAGE

Reagent Handling

- R1 Ready for use.
- R2 Ready for use.
- Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.

  CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration that could impact results.

REAGENT HANDLING AND STORAGE (Continued)

Reagent Storage

- Unopened reagents are stable until the expiration date when stored at 2 to 8°C.
- Reagent stability is 60 days if the reagent is uncapped and onboard.

Indications of Deterioration

Instability or deterioration should be suspected if there are visible signs of leakage, extreme turbidity, microbial growth, if calibration does not meet the appropriate package insert and/or **ARCHITECT System Operations Manual** criteria, or if controls do not meet the appropriate criteria.

WARNINGS AND PRECAUTIONS

Precautions for Users

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[1] Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.
- This product contains sodium azide; for a specific listing, refer to the REAGENTS section of this package insert. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
  NOTE: Refer to *Section 8* of the **ARCHITECT System Operations Manual** for proper handling and disposal of reagents containing sodium azide.

SPECIMEN COLLECTION AND HANDLING

Suitable Specimens

- **Serum:** Use serum collected by standard venipuncture techniques into plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. When processing samples, separate serum from blood cells or gel according to the specimen collection tube manufacturer's instructions.

  Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous test results.
- **Plasma:** Use plasma collected by standard venipuncture techniques into plastic tubes. Acceptable anticoagulants are lithium heparin (with or without gel barrier), sodium heparin, and EDTA. Ensure centrifugation is adequate to remove platelets. When processing samples, separate plasma from blood cells or gel according to the specimen collection tube manufacturer's instructions.

NOTE: Glass tubes were not tested.

For total sample volume requirements, refer to the ASSAY PARAMETERS section of this package insert and *Section 5* of the **ARCHITECT System Operations Manual**.

Specimen Storage

Temperature	Maximum Storage	Bibliographic Reference
20 to 25°C	15 days	5
2 to 8°C	2 months	5, 6
-20°C	5 years	5

Guder et al.[5] suggest storage of frozen specimens at -20°C for no longer than the time interval cited above. However, limitations of laboratory equipment make it necessary in practice for clinical laboratories to establish a range around -20°C for specimen storage. This temperature range may be established from either the freezer manufacturer's specifications or your laboratory standard operating procedure(s) for specimen storage.

NOTE: Stored specimens must be inspected for particulates. If present, mix and centrifuge the specimen to remove particulates prior to testing.

PROCEDURE

Materials Provided

REF 6K26-30 MULTIGENT CRP Vario Kit
REF 6K26-41 MULTIGENT CRP Vario Kit

Materials Required but not Provided

- REF 6K26-10 MULTIGENT CRP Calibrator Set
- REF 6K26-14 MULTIGENT CRP Calibrator HS
- REF 6K26-12 MULTIGENT CRP Calibrator WR
- REF 6K26-21 MULTIGENT CRP Control HS
- REF 6K32-20 Immuno Control 1 (Not available in the US)
- REF 6K32-21 Immuno Control 2 (Not available in the US)
- Saline (0.85% to 0.90% NaCl) for specimens that require dilution

Assay Procedure

For a detailed description of how to run an assay on the ARCHITECT *c* Systems, refer to *Section 5* of the **ARCHITECT System Operations Manual**.

Specimen Dilution Procedure

The ARCHITECT *c* Systems have automatic dilution features; refer to *Section 2* of the **ARCHITECT System Operations Manual** for additional information.

Serum and Plasma: Specimens with CRP values exceeding the linearity are flagged and may be diluted by following either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

If using the Automated Dilution Protocol, the system performs a dilution of the specimen and automatically corrects the concentration by multiplying the result by the appropriate dilution factor. The dilution for each method is listed below.

Method	Dilution
High sensitivity	1:10
Standard	1:5
Wide range	1:5

Manual Dilution Procedure

- Use saline (0.85% to 0.90% NaCl) to dilute the sample.
- The operator must enter the dilution factor in the patient or control order screen. The system uses this dilution factor to automatically correct the concentration by multiplying the result by the entered factor.
- If the operator does not enter the dilution factor, the result must be multiplied by the appropriate dilution factor before reporting the result.

NOTE: If a diluted sample result is flagged indicating it is less than the linear low limit, do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to *Section 5* of the **ARCHITECT System Operations Manual**.

CALIBRATION

NOTE: The MULTIGENT CRP Vario assay must be calibrated using the individual levels listed in the ASSAY PARAMETERS. Refer to the parameters for the High Sensitivity (CRP15), Standard (CRP32), and Wide Range (CRP48) methods and the MULTIGENT CRP Calibrator package insert specific for the method used in your laboratory.

Calibration is stable for approximately 15 days (360 hours) and is required with each change in reagent lot number. Verify calibration curve with at least two levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

For a detailed description of how to calibrate an assay, refer to *Section 6* of the **ARCHITECT System Operations Manual**.

Standardization

For information on calibrator standardization, refer to the MULTIGENT CRP Calibrator package insert specific for the method used in your laboratory.

QUALITY CONTROL

As appropriate, refer to your laboratory standard operating procedure(s) and/or quality assurance plan for additional quality control requirements and potential corrective actions:

- Two levels of controls (normal and abnormal) are to be run every 24 hours.
- If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
- If quality control results do not meet the acceptance criteria defined by your laboratory, patient values may be suspect. Follow the established quality control procedures for your laboratory. Recalibration may be necessary.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

RESULTS

Refer to *Appendix C* of the **ARCHITECT System Operations Manual** for information on results calculations.

To convert results from mg/dL to mg/L, multiply mg/dL by 10.[7]

Representative performance data are given in the EXPECTED VALUES and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert. Results obtained in individual laboratories may vary.

LIMITATIONS OF THE PROCEDURE

The following are limitations on the use of the High Sensitivity CRP per CDC/AHA recommendations:[8]

- Screening the entire adult population is not recommended.
- CRP is not a substitute for traditional cardiovascular risk factors.
- Acute coronary syndrome management should not depend on CRP measurements.
- Patients with persistently unexplained CRP levels above 10 mg/dL (10 mg/L) should be evaluated for noncardiovascular etiologies.
- Secondary prevention measures should not depend on CRP.
- Serial measurements of CRP should not be used to monitor treatment.
- The average of two CRP results, repeated optimally two weeks apart, should be used on metabolically stable patients.

In very rare cases gammopathy, particularly of the monoclonal IgM type (e.g., Waldenström macroglobulinemia), may cause unreliable results.

Refer to the SPECIMEN COLLECTION AND HANDLING and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

EXPECTED VALUES

Reference Range

	Range (mg/dL)	Range (mg/L)
Serum and plasma[9]	≤ 0.5	≤ 5

Schlebusch et al.[10] have published pediatric reference ranges.

CRP is an acute phase protein whose concentration rises non-specifically in response to inflammation. CRP values should not be interpreted without a complete clinical evaluation. Follow-up testing of patients with elevated values is recommended in order to help rule out a recent response to undetected infection or tissue injury. It is recommended that each laboratory establish its own expected range. For diagnostic purposes, the patient's medical history and all other clinical findings should be considered when evaluating CRP results.

SPECIFIC PERFORMANCE CHARACTISTICS

Reportable Range

The reportable range for MULTIGENT CRP Vario is:

High Sensitivity Method	0.01 to 16.00 mg/dL (0.1 to 160 mg/L)
Standard Method	0.02 to 32.00 mg/dL (0.2 to 320 mg/L)
Wide Range	0.02 to 48.00 mg/dL (0.2 to 480 mg/L)

All three methods were tested for prozone up to a CRP concentration of 100 mg/dL (1,000 mg/L). No prozone effect was observed within the linear range of the assay. At 100 mg/dL (1,000 mg/L) the observed result was correctly flagged as above the linearity of the assay.

Limit of Quantitation (LOQ)

The LOQ is the analyte concentration at which the CV = 20%. The limit of quantification for MULTIGENT CRP Vario is:

High Sensitivity Method	0.01 mg/dL (0.1 mg/L)
Standard and Wide Range Methods	0.02 mg/dL (0.2 mg/L)

Interfering Substances

Interference studies were conducted using an acceptance criteria of ± 5% deviation from the target value. No interference was observed at the concentrations below.

Interfering Substance	Interferent Concentration
Bilirubin, conjugated and fetal	30 mg/dL (513 µmol/L)
Hemoglobin	500 mg/dL (5 g/L)
Intralipid	1,500 mg/dL (15 g/L)
Rheumatoid factor	550 IU/mL (550 kU/L)

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ARCHITECT *c* Systems Assay Parameters

CRP Vario (Wide Range Method) Serum/Plasma: Conventional and SI Units

Configure assay parameters — General

● General | O Calibration | O SmartWash | O Results | O Interpretation

Assay: CRP48 Type: Photometric Version: †
Number: 2975
Run controls for onboard reagents by:* Lot

● Reaction definition | O Reagent / Sample | O Validity checks

Reaction mode: Rate up
Primary / Secondary — Read times
Wavelength: 572 / None — Main: 20 – 25
Last required read: 28 — Flex: ___ – ___
Absorbance range: 0.7000 – 3.2000 — Color correction: ___ – ___
Sample blank type: None

O Reaction definition | ● Reagent / Sample | O Validity checks

						R1	R2
Reagent: CRP0S					Reagent volume:	120	120
Diluent: Saline					Water volume:	___	___
Diluent dispense mode: Type 0					Dispense mode:	Type 0	Type 0
Dilution name	Sample	Diluted sample	Diluent	Water	Dilution factor		Default dilution
STANDARD	2.0	___	___	___	1:1.00		●
DIL 1	20.0	2.0	80	___	1:5.00		O
___	___	___	___	___	___		O

O Reaction definition | O Reagent / Sample | ● Validity checks

Reaction check: None

Rate linearity %: ___

Configure assay parameters — Calibration

O General | ● Calibration | O SmartWash | O Results | O Interpretation

Assay: CRP48 Calibration method: Spline

● Calibrators | O Volumes | O Intervals | O Validity checks

Calibrator set:	Calibrator level:	(Short name)‡‡	Concentration‡ (mg/dL)	Concentration‡ (mg/L)
48CRP	Blank: Water		0.00	0.0
	Cal 1:	48CRP1 (CRP05)	0.50	5.0
Replicates: 2 [Range 1 - 3]	Cal 2:	48CRP2 (CRP10)	1.00	10.0
	Cal 3:	48CRP3 (CRP20)	2.00	20.0
	Cal 4:	48CRP4 (CRP40)	4.00	40.0
	Cal 5:	48CRP5 (CRP160)	16.00	160.0
	Cal 6:	48CRP6 (CRPWR)	48.00	480.0

O Calibrators | ● Volumes | O Intervals | O Validity checks

Calibrator: 48CRP

	Calibrator level	Sample	Diluted sample	Diluent	Water
Blank:	Water	2.0	___	___	___
Cal 1:	48CRP1	2.0	___	___	___
Cal 2:	48CRP2	4.0	___	___	___
Cal 3:	48CRP3	5.0	___	___	___
Cal 4:	48CRP4	5.0	___	___	___
Cal 5:	48CRP5	3.0	___	___	___
Cal 6:	48CRP6	2.0	___	___	___

O Calibrators | O Volumes | ● Intervals | O Validity checks

Calibration intervals:
Full interval: 360 (hours)
Calibration type:
Adjust type: None

O Calibrators | O Volumes | O Intervals | ● Validity checks

Blank absorbance range: -0.0500 – 0.0500
Span: Blank – Blank
Span absorbance range: ____ – ____
Expected cal factor: 0.00
Expected cal factor tolerance %: 0

Configure assay parameters — SmartWash

O General | O Calibration | ● SmartWash | O Results | O Interpretation

Assay: CRP48

COMPONENT	REAGENT / ASSAY	WASH	Volume	Replicates
Cuvette	Trig**	10% Detergent B	345	

** Not required for ARCHITECT software version 7.00 and above.

CRP Vario (Wide Range Method) Serum/Plasma: Conventional Units

Configure assay parameters — Results

O General | O Calibration | O SmartWash | ● Results | O Interpretation

Assay: CRP48 — Assay number: 2975
Dilution default range: — Result units: mg/dL
Low-Linearity: 0.02
High-Linearity: 48.00
Gender and age specific ranges:

GENDER	AGE (UNITS)	NORMAL	EXTREME
Either	0 – 130 (Y)	0.00 – 0.50	

Configure result units

Assay: CRP48
Version: †
Result units: mg/dL
Decimal places: 2 [Range 0 – 4]
Correlation factor: 1.0000
Intercept: 0.0000

CRP Vario (Wide Range Method) Serum/Plasma: SI Units

Configure assay parameters — Results

O General | O Calibration | O SmartWash | ● Results | O Interpretation

Assay: CRP48 — Assay number: 2975
Dilution default range: — Result units: mg/L
Low-Linearity: 0.2
High-Linearity: 480.0
Gender and age specific ranges:

GENDER	AGE (UNITS)	NORMAL	EXTREME
Either	0 – 130 (Y)	0.0 – 5.0	

Configure result units

Assay: CRP48
Version: †
Result units: mg/L
Decimal places: 1 [Range 0 – 4]
Correlation factor: 1.0000
Intercept: 0.0000

† Due to differences in instrument systems and unit configurations, version numbers may vary.
* Parameter is available in ARCHITECT software version 7.00 and above.
‡ Displays the number of decimal places defined in the decimal place field. In ARCHITECT software version 5.00 and above, these values are defined on the Configure calibrator set screen.
‡‡ Short name does not display on instrument screen.

SPECIFIC PERFORMANCE CHARACTISTICS (Continued)

Precision

The precision of the MULTIGENT CRP Vario assay is ≤ 6% Total CV. Studies were performed using CLSI protocol NCCLS EP5-A.[11] Representative results in mg/L are summarized below.

CRP High Sensitivity Method

		Level 1	Level 2	Level 3	Level 4
N		40	40	40	40
Mean (mg/L)		0.462	4.92	11.35	45.88
Within Run	SD	0.019	0.035	0.148	0.298
	%CV	4.00	0.72	1.30	0.65
Between Run	SD	0.011	0.024	0.109	0.120
	%CV	2.38	0.49	0.96	0.26
Total	SD	0.022	0.043	0.183	0.389
	%CV	4.66	0.87	1.62	0.85

NOTE: % CV was calculated prior to rounding Mean and SD.

CRP Standard Method

		Level 1	Level 2	Level 3	Level 4
N		40	40	40	40
Mean (mg/L)		5.10	18.30	73.30	319.40
Within Run	SD	0.10	0.11	0.37	2.08
	%CV	1.97	0.59	0.50	0.65
Between Run	SD	0.04	0.12	0.14	1.12
	%CV	0.78	0.65	0.19	0.35
Total	SD	0.11	0.19	0.40	2.70
	%CV	2.15	1.04	0.54	0.85

NOTE: % CV was calculated prior to rounding Mean and SD.

CRP Wide Range Method

		Level 1	Level 2	Level 3	Level 4
N		40	40	40	40
Mean (mg/L)		5.20	18.40	90.20	288.00
Within Run	SD	0.06	0.19	0.69	3.32
	%CV	1.16	0.99	0.77	1.24
Between Run	SD	0.05	0.08	0.76	3.35
	%CV	0.98	0.44	0.84	1.25
Total	SD	0.1	0.22	1.25	4.92
	%CV	1.91	1.17	1.39	1.83

Method Comparison

Serum results from the MULTIGENT CRP Vario methods on the AEROSET System were compared with the results from a commercially available nephelometric methodology.

Serum results from the MULTIGENT CRP Vario methods on an ARCHITECT *c* System were compared with the results on the AEROSET System.

For the MULTIGENT CRP High Sensitivity method only, serum results from an ARCHITECT *c* System and the AEROSET System were also compared with the results from a commercially available turbidimetric methodology.

Method comparison data are presented in mg/L.

CRP High Sensitivity Method	AEROSET vs. Nephelometer	ARCHITECT vs. AEROSET
N	45	45
Y - Intercept (95% CI*)	-0.24 to 0.54	-0.33 to 0.45
Correlation Coefficient	0.9994	0.9994
Slope (95% CI*)	0.985 to 1.006	0.975 to 0.996
Standard Error of the Estimate	0.96	0.96
Range (mg/L)	1.0 to 104.0	1.1 to 103.3

* CI = Confidence Interval

CRP High Sensitivity Method	AEROSET vs. Turbidimetric Method	ARCHITECT vs. Turbidimetric Method
N	55	55
Y - Intercept (95% CI*)	-0.01 to 0.07	-0.02 to 0.10
Correlation Coefficient	0.9995	0.9990
Slope (95% CI*)	0.992 to 1.009	1.001 to 1.035
Standard Error of the Estimate	0.10	0.14
Range (mg/L)	0.2 to 13.6	0.2 to 13.6

* CI = Confidence Interval

Method Comparison (Continued)

CRP Standard Method	AEROSET vs. Nephelometer	ARCHITECT vs. AEROSET
N	54	54
Y - Intercept (95% CI*)	-0.68 to 0.50	-0.64 to 0.46
Correlation Coefficient	0.9996	0.9997
Slope (95% CI*)	1.004 to 1.020	0.977 to 0.991
Standard Error of the Estimate	1.64	1.58
Range (mg/L)	1.0 to 219.0	0.6 to 223

* CI = Confidence Interval

CRP Wide Range Method	AEROSET vs. Nephelometer	ARCHITECT vs. AEROSET
N	59	59
Y - Intercept (95% CI*)	-1.26 to 1.53	-1.44 to 2.04
Correlation Coefficient	0.9989	0.9982
Slope (95% CI*)	1.023 to 1.051	0.968 to 0.999
Standard Error of the Estimate	4.10	5.28
Range (mg/L)	1.0 to 286.0	0.7 to 301.2

* CI = Confidence Interval

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TRADEMARKS

6K263041_IFU_01_EN

ARCHITECT cSystems Assay Parameters

CRP Vario (High Sensitivity Method) Serum/Plasma: Conventional and SI Units

Configure assay parameters — General

● General | O Calibration | O SmartWash | O Results | O Interpretation

Assay: CRP16 Type: Photometric Version: †
Number: 2974
Run controls for onboard reagents by:* Lot

● Reaction definition | O Reagent / Sample | O Validity checks

Reaction mode: Rate up

	Primary	Secondary		Read times
Wavelength:	572	/ None	Main:	20 – 25
Last required read:	26		Flex:	___ – ___
Absorbance range:	0.7000 – 3.2000		Color correction:	___ – ___
Sample blank type:	None			

O Reaction definition | ● Reagent / Sample | O Validity checks

						R1	R2
Reagent:	CRP0S			Reagent volume:		100	100
Diluent:	Saline			Water volume:		___	___
Diluent dispense mode:	Type 0			Dispense mode:		Type 0	Type 0

Dilution name	Sample	Diluted sample	Diluent	Water	Dilution factor	Default dilution
STANDARD :	4.0	___	___	___ =	1:1.00	●
DIL 1 :	10.0	4.0	90	___ =	1:10.00	O
_____ :	___	___	___	___ =	___	O

O Reaction definition | O Reagent / Sample | ● Validity checks

Reaction check: None

Rate linearity %: ___

Configure assay parameters — Calibration

O General | ● Calibration | O SmartWash | O Results | O Interpretation

Assay: CRP16 Calibration method: Spline

● Calibrators | O Volumes | O Intervals | O Validity checks

Calibrator set: 16CRP
Replicates: 2 [Range 1 - 3]

Calibrator level:	(Short name)‡‡	Concentration‡ (mg/dL)	Concentration‡ (mg/L)
Blank: Water		0.00	0.00
Cal 1: 16CRP1	(CRP1S)	0.25	2.50
Cal 2: 16CRP2	(CRP5)	0.50	5.00
Cal 3: 16CRP3	(CRP10)	1.00	10.00
Cal 4: 16CRP4	(CRP20)	2.00	20.00
Cal 5: 16CRP5	(CRP80)	8.00	80.00
Cal 6: 16CRP6	(CRP160)	16.00	160.00

O Calibrators | ● Volumes | O Intervals | O Validity checks

Calibrator: 16CRP

Calibrator level	Sample	Diluted sample	Diluent	Water
Blank: Water	4.0	___	___	___
Cal 1: 16CRP1	4.0	___	___	___
Cal 2: 16CRP2	4.0	___	___	___
Cal 3: 16CRP3	4.0	___	___	___
Cal 4: 16CRP4	4.0	___	___	___
Cal 5: 16CRP5	4.0	___	___	___
Cal 6: 16CRP6	4.0	___	___	___

O Calibrators | O Volumes | ● Intervals | O Validity checks

Calibration intervals:
Full interval: 360 (hours)
Calibration type:
Adjust type: None

O Calibrators | O Volumes | O Intervals | ● Validity checks

Blank absorbance range: -0.0500 – 0.0500
Span: Blank – Blank
Span absorbance range: _____ – _____
Expected cal factor: 0.00
Expected cal factor tolerance %: 0

Configure assay parameters — SmartWash

O General | O Calibration | ● SmartWash | O Results | O Interpretation

Assay: CRP16

COMPONENT	REAGENT / ASSAY	WASH	Volume	Replicates
Cuvette	Trig**	10% Detergent B	345	

** Not required for ARCHITECT software version 7.00 and above.

CRP Vario (High Sensitivity Method) Serum/Plasma: Conventional Units

Configure assay parameters — Results

O General | O Calibration | O SmartWash | ● Results | O Interpretation

Assay: CRP16 Assay number: 2974
Dilution default range: Result units: mg/dL
Low-Linearity: 0.01
High-Linearity: 16.00
Gender and age specific ranges:

GENDER	AGE (UNITS)	NORMAL	EXTREME
Either	0 – 130 (Y)	0.00 – 0.50	

Configure result units

Assay: CRP16
Version: †
Result units: mg/dL
Decimal places: 2 [Range 0 – 4]
Correlation factor: 1.0000
Intercept: 0.0000

CRP Vario (High Sensitivity Method) Serum/Plasma: SI Units

Configure assay parameters — Results

O General | O Calibration | O SmartWash | ● Results | O Interpretation

Assay: CRP16 Assay number: 2974
Dilution default range: Result units: mg/L
Low-Linearity: 0.10
High-Linearity: 160.00
Gender and age specific ranges:

GENDER	AGE (UNITS)	NORMAL	EXTREME
Either	0 – 130 (Y)	0.00 – 5.00	

Configure result units

Assay: CRP16
Version: †
Result units: mg/L
Decimal places: 2 [Range 0 – 4]
Correlation factor: 1.0000
Intercept: 0.0000

† Due to differences in instrument systems and unit configurations, version numbers may vary.
* Parameter is available in ARCHITECT software version 7.00 and above.
‡ Displays the number of decimal places defined in the decimal place field. In ARCHITECT software version 5.00 and above, these values are defined on the Configure calibrator set screen.
‡‡ Short name does not display on instrument screen.

ARCHITECT cSystems Assay Parameters

CRP Vario (Standard Method) Serum/Plasma: Conventional and SI Units

Configure assay parameters — General

● General | O Calibration | O SmartWash | O Results | O Interpretation

Assay: CRP32 Type: Photometric Version: †
Number: 2973
Run controls for onboard reagents by:* Lot

● Reaction definition | O Reagent / Sample | O Validity checks

Reaction mode: Rate up

	Primary	Secondary		Read times
Wavelength:	572	/ None	Main:	20 – 25
Last required read:	26		Flex:	___ – ___
Absorbance range:	0.7000 – 3.2000		Color correction:	___ – ___
Sample blank type:	None			

O Reaction definition | ● Reagent / Sample | O Validity checks

						R1	R2
Reagent:	CRP0S			Reagent volume:		100	100
Diluent:	Saline			Water volume:		___	___
Diluent dispense mode:	Type 0			Dispense mode:		Type 0	Type 0

Dilution name	Sample	Diluted sample	Diluent	Water	Dilution factor	Default dilution
STANDARD :	2.0	___	___	___ =	1:1.00	●
DIL 1 :	20.0	2.0	80	___ =	1:5.00	O
_____ :	___	___	___	___ =	___	O

O Reaction definition | O Reagent / Sample | ● Validity checks

Reaction check: None

Rate linearity %: ___

Configure assay parameters — Calibration

O General | ● Calibration | O SmartWash | O Results | O Interpretation

Assay: CRP32 Calibration method: Spline

● Calibrators | O Volumes | O Intervals | O Validity checks

Calibrator set: 32CRP
Replicates: 2 [Range 1 - 3]

Calibrator level:	(Short name)‡‡	Concentration‡ (mg/dL)	Concentration‡ (mg/L)
Blank: Water		0.00	0.0
Cal 1: 32CRP1	(CRP05)	0.50	5.0
Cal 2: 32CRP2	(CRP10)	1.00	10.0
Cal 3: 32CRP3	(CRP20)	2.00	20.0
Cal 4: 32CRP4	(CRP40)	4.00	40.0
Cal 5: 32CRP5	(CRP160)	16.00	160.0
Cal 6: 32CRP6	(CRP320)	32.00	320.0

O Calibrators | ● Volumes | O Intervals | O Validity checks

Calibrator: 32CRP

Calibrator level	Sample	Diluted sample	Diluent	Water
Blank: Water	2.0	___	___	___
Cal 1: 32CRP1	2.0	___	___	___
Cal 2: 32CRP2	2.0	___	___	___
Cal 3: 32CRP3	2.0	___	___	___
Cal 4: 32CRP4	2.0	___	___	___
Cal 5: 32CRP5	2.0	___	___	___
Cal 6: 32CRP5	2.0	___	___	___

O Calibrators | O Volumes | ● Intervals | O Validity checks

Calibration intervals:
Full interval: 360 (hours)
Calibration type:
Adjust type: None

O Calibrators | O Volumes | O Intervals | ● Validity checks

Blank absorbance range: -0.0500 – 0.0500
Span: Blank – Blank
Span absorbance range: _____ – _____
Expected cal factor: 0.00
Expected cal factor tolerance %: 0

Configure assay parameters — SmartWash

O General | O Calibration | ● SmartWash | O Results | O Interpretation

Assay: CRP32

COMPONENT	REAGENT / ASSAY	WASH	Volume	Replicates
Cuvette	Trig**	10% Detergent B	345	

** Not required for ARCHITECT software version 7.00 and above.

CRP Vario (Standard Method) Serum/Plasma: Conventional Units

Configure assay parameters — Results

O General | O Calibration | O SmartWash | ● Results | O Interpretation

Assay: CRP32 Assay number: 2973
Dilution default range: Result units: mg/dL
Low-Linearity: 0.02
High-Linearity: 32.00
Gender and age specific ranges:

GENDER	AGE (UNITS)	NORMAL	EXTREME
Either	0 – 130 (Y)	0.00 – 0.50	

Configure result units

Assay: CRP32
Version: †
Result units: mg/dL
Decimal places: 2 [Range 0 – 4]
Correlation factor: 1.0000
Intercept: 0.0000

CRP Vario (Standard Method) Serum/Plasma: SI Units

Configure assay parameters — Results

O General | O Calibration | O SmartWash | ● Results | O Interpretation

Assay: CRP32 Assay number: 2973
Dilution default range: Result units: mg/L
Low-Linearity: 0.2
High-Linearity: 320.0
Gender and age specific ranges:

GENDER	AGE (UNITS)	NORMAL	EXTREME
Either	0 – 130 (Y)	0.0 – 5.0	

Configure result units

Assay: CRP32
Version: †
Result units: mg/L
Decimal places: 1 [Range 0 – 4]
Correlation factor: 1.0000
Intercept: 0.0000

† Due to differences in instrument systems and unit configurations, version numbers may vary.
* Parameter is available in ARCHITECT software version 7.00 and above.
‡ Displays the number of decimal places defined in the decimal place field. In ARCHITECT software version 5.00 and above, these values are defined on the Configure calibrator set screen.
‡‡ Short name does not display on instrument screen.

Ferritin protocol

ARCHITECT
SYSTEM

en
Ferritin
REF 7K59
G3-0038/R06
B7K590

Read Highlighted Changes
Revised July 2012

Ferritin

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	REAGENT LOT	Reagent Lot
IVD	*In Vitro* Diagnostic Medical Device	SN	Serial Number
		REACTION VESSELS	Reaction Vessels
LOT	Lot Number	SAMPLE CUPS	Sample Cups
	Expiration Date	SEPTUM	Septum
2°C 8°C	Store at 2-8°C	REPLACEMENT CAPS	Replacement Caps
		GTIN	Global Trade Item Number
	Consult instructions for use	PRODUCT OF IRELAND	Product of Ireland
	Manufacturer	INFORMATION FOR USA ONLY	Information needed for United States of America only

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

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NAME

ARCHITECT Ferritin

INTENDED USE

The ARCHITECT Ferritin assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of ferritin in human serum and plasma.

SUMMARY AND EXPLANATION OF TEST

Ferritin is a high-molecular weight iron-containing protein that functions in the body as an iron storage compound. Each ferritin molecule is thought to consist of a spherical protein shell of molecular weight about 460,000 daltons made up of 24 subunits with a variable amount of iron as a core of ferricoxide-phosphate.[1,2] It has been demonstrated that the ferritin molecule, when fully saturated, may consist of over 20% iron by weight.[2]

Approximately 25% of the iron in a normal adult is present in various storage forms.[3] About two-thirds of the iron stores in the human body exist in the form of ferritin. The remaining iron stores are contained in insoluble hemosiderin, which most likely represents a form of denatured ferritin.[4]

The availability of sensitive methods for measuring serum ferritin have significantly advanced the ability to detect iron deficiency and overload. Since iron deficiency is present before the onset of anemia, detection of an iron depleted state is important for the control of nutritional anemia. The clinical assessment of iron stores has historically relied on the determination of serum iron, total iron-binding capacity (TIBC) and percent transferrin (ratio of serum iron and TIBC) or direct examination of bone marrow.

The estimation of stainable iron in the bone marrow is the traditional method for assessing body iron stores. This biopsy method provides a sensitive index of iron deficiency but has the disadvantage of being subjective and semiquantitative. Low hemoglobin concentration is the most readily available sign of anemia, but a significant fall in circulating hemoglobin cannot be detected until the final stage of iron deficiency anemia. Serum iron, TIBC and percent transferrin saturation do not distinguish iron deficiency as a progressive disease. Also, these measurements are affected by diurnal variation and may not discriminate between depleted iron stores and conditions associated with defective reticuloendothelial release of iron (e.g., anemia of chronic disease).[3] Recent literature suggests that ferritin provides a more sensitive, specific and reliable measurement for determining iron deficiency at an early stage.[9] In patients being given iron orally, serum ferritin measurements have been shown to be useful for monitoring the reaccumulation of iron stores and determining when therapy can be discontinued.[10] In chronic inflammatory disorders, infections, and in chronic renal failure, there is a disproportionate increase in serum ferritin levels in relation to iron stores. The correlation of serum ferritin to body iron stores still exists, however, it is set at a higher level of serum ferritin.[7,8,10] Numerous studies in the literature demonstrate the usefulness and necessity of serum ferritin measurements in combination with other parameters in determining the rate and degree of body iron overload in such disorders as thalassemia, sideroblastic anemia and in determining the response of patients treated with iron chelating agents.[5,6] Specifically, the combined use of serum ferritin levels and mean corpuscular volume (MCV) has made differentiation between iron deficiency, beta-thalassemia trait and normal subjects possible at a very high level of accuracy.[11,12]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Ferritin assay is a two-step immunoassay to determine the presence of ferritin in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex.

In the first step, sample and anti-ferritin coated paramagnetic microparticles are combined. Ferritin present in the sample binds to the anti-ferritin coated microparticles. After washing, anti-ferritin acridinium labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of ferritin in the sample and the RLUs detected by the ARCHITECT *i* optical system.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT Ferritin Reagent Kit (7K59)

- **MICROPARTICLES** 1 or 4 Bottle(s) (6.6 mL/27.0 mL) Anti-Ferritin (mouse, monoclonal) coated Microparticles in TRIS buffer with protein (mouse and bovine) stabilizers. Preservative: antimicrobial agent.
- **CONJUGATE** 1 or 4 Bottle(s) (5.9 mL/26.3 mL) Anti-Ferritin (rabbit, polyclonal) acridinium labeled Conjugate in MES buffer with protein (bovine) stabilizers. Minimum concentration: 75 ng/mL. Preservative: antimicrobial agent.

Assay Diluent

ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50)

- **MANUAL DILUENT** 1 Bottle (100 mL) ARCHITECT *i* Multi-Assay Manual Diluent containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

NOTE: Bottle and volume varies based on order.

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.[13] Biosafety Level 2[14] or other appropriate biosafety practices[15,16] should be used for materials that contain or are suspected of containing infectious agents.
- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not mix reagents from different reagent kits.**
- Prior to loading the ARCHITECT Ferritin Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT Ferritin Reagent Kit must be stored at 2-8°C and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT Ferritin Reagent Kit may be stored on-board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking on-board time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** After reagents are removed from the system, you must initiate a scan to update the on-board stability timer.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT Ferritin assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* Assay CD-ROM prior to performing the assay. For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in serum separator tubes) or plasma collected in tripotassium EDTA and lithium heparin may be used in the ARCHITECT Ferritin assay. Individual plasma concentration values may differ from serum by more than 10%. Samples in tripotassium EDTA may give values below those of serum, while samples collected in lithium heparin may give values greater than serum values. Other anticoagulants have not been verified for use with the ARCHITECT Ferritin assay. Follow the tube manufacturer's processing instructions for serum or plasma collection tubes.
- When serial specimens are being evaluated, the same type of specimen should be used throughout the study.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT Ferritin assay.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- For optimal results, serum and plasma specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- If testing will be delayed more than 24 hours, remove serum or plasma from the clot, serum separator or red blood cells. Specimens may be stored for up to 7 days at 2-8°C prior to being tested. If testing will be delayed more than 7 days, specimens should be frozen at -10°C or colder. Specimens stored frozen at -10°C or colder for 12 months showed no performance difference.
- Multiple freeze-thaw cycles of specimens should be avoided. Specimens must be mixed THOROUGHLY after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens may be shipped frozen on dry ice. Prior to shipment, it is recommended that specimens be removed from the clot, serum separator or red blood cells.

PROCEDURE

Materials Provided

- 7K59 ARCHITECT Ferritin Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT *i* Assay CD-ROM
- 7K59-01 ARCHITECT Ferritin Calibrators
- 7K59-10 ARCHITECT Ferritin Controls
- 7D82-50 ARCHITECT *i* Multi-Assay Manual Diluent
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

Assay Procedure

- Before loading the ARCHITECT Ferritin Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment:
  - Invert the microparticle bottle 30 times.
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
- Order tests.
- Load the ARCHITECT Ferritin Reagent Kit on the ARCHITECT *i* System. Verify that all necessary assay reagents are present. Ensure that septums are present on all reagent bottles.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test.
  - Priority: 70 µL for the first Ferritin test plus 20 µL for each additional Ferritin test from the same sample cup
  - ≤ 3 hours onboard: 150 µL for the first Ferritin test plus 20 µL for each additional Ferritin test from the same sample cup
  - > 3 hours onboard: additional sample volume is required. Refer to the ARCHITECT System Operations Manual, Section 5 for information on sample evaporation and volumes.

- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- ARCHITECT Ferritin Calibrators and ARCHITECT Ferritin Controls should be mixed by gentle inversion prior to use.
- To obtain the recommended volume requirements for the ARCHITECT Ferritin Calibrators and ARCHITECT Ferritin Controls, hold the bottles **vertically** and dispense 4 drops of each calibrator or 3 drops of each control into each respective sample cup.
- Load samples
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5
- Press RUN. The ARCHITECT *i* System performs the following function:
  - Moves the sample to the aspiration point
  - Loads a reaction vessel (RV) into the process path
  - Aspirates and transfers sample into the RV
  - Advances the RV one position and transfers microparticles into the RV
  - Mixes, incubates and washes the reaction mixture
  - Adds conjugate to the RV
  - Mixes, incubates and washes the reaction mixture
  - Adds Pre-Trigger and Trigger Solutions
  - Measures chemiluminescent emission to determine the quantity of ferritin in the sample
  - Aspirates contents of RV to liquid waste and unloads RV to solid waste
  - Calculates the result
- For information on ordering patient specimens, calibrators and controls, and general operating procedures refer to the ARCHITECT System Operations Manual, Section 5.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Specimens with a ferritin value exceeding 2000 ng/mL are flagged with the code " >2000" and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

- If using the Automated Dilution Protocol, the system performs a 1:20 dilution of the specimen. The system will use the dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.
- Manual dilutions should be performed as follows:
  - The suggested dilution for ferritin is 1:20.
  - For a 1:20 dilution, add 20 µL of the patient specimen to 380 µL of ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
  - The operator must enter the dilution factor in the patient or control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result. The dilution should be performed so that the diluted result reads greater than 80 ng/mL. If the diluted result reads less than 80 ng/mL, the sample should be retested undiluted.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT Ferritin calibration, test ARCHITECT Ferritin Calibrators 1 and 2 in duplicate. A single sample of all levels of ARCHITECT Ferritin controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the package insert for controls. Calibrators should be priority loaded.
- Calibration Range: 0 - 1000 ng/mL.
- The assay protocol extends the assay range to 0 - 2000 ng/mL.
- Once an ARCHITECT Ferritin calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used
  - Controls are out of range
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Ferritin assay is a single sample of all control levels tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. Ensure that assay control values are within the concentration ranges specified in the package insert for controls.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Ferritin assay belongs to method group 1. For the analytical sensitivity calculation, follow method group 1 and divide the result by 2.

RESULTS

The ARCHITECT Ferritin assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, X weighted) to generate a calibration curve.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- If the Ferritin results are inconsistent with clinical evidence, additional testing is supposed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.[17,18] Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[19] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

EXPECTED VALUES

Evaluation of serum specimens from 32 normal males and 60 normal females with the ARCHITECT Ferritin assay yielded the following results.

Normal Range Summary	No. of Subjects	Median (ng/mL)	Central 95% Interval (ng/mL)
Males	32	75.62	21.81 - 274.66
Females	60	39.42	4.63 - 204.00

These individuals were determined to be normal based on the AxSYM Ferritin Assay. It is recommended that each laboratory establish its own range, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Ferritin levels below 10 ng/mL have been reported as indicative of iron deficiency anemia.[20,21] There are patients with iron deficiency anemia who have elevated or normal ferritin levels because of other causes, such as hepatocellular disease or iron therapy.[4,7]

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Ferritin assay is designed to have a precision of ≤ 9 total %CV for concentrations within the range of the low, medium and high control (Panel Members 1-3). A study based on guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-T2 was performed for the ARCHITECT Ferritin assay.[22] A three member buffered protein based panel (1, 2, and 3) and a three member reconstituted processed human serum panel (4, 5, and 6) were assayed in replicates of two at two separate times per day, for 20 days on one instrument using two lots of reagents and a single calibration for each reagent lot. Data from this study are summarized in the following table.

Panel Member	Reagent Lot	Instrument	n	Mean Conc. Value (ng/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
1	1	1	80	20.33	0.723	3.6	0.968	4.8
1	2	1	80	19.08	0.818	4.3	1.217	6.4
2	1	1	80	148.07	5.760	3.9	8.417	5.7
2	2	1	80	145.38	5.303	3.6	8.096	5.6
3	1	1	80	391.47	15.574	4.0	21.593	5.5
3	2	1	80	387.61	13.012	3.4	21.703	5.6
4	1	1	80	421.13	15.847	3.8	25.080	6.0
4	2	1	80	408.61	14.353	3.5	21.504	5.3
5	1	1	80	155.03	5.338	3.4	14.962	9.7
5	2	1	80	149.89	5.226	3.5	15.514	10.3
6	1	1	80	40.52	1.286	3.2	4.500	11.1
6	2	1	80	39.22	1.336	3.4	4.600	11.7

Recovery

The ARCHITECT Ferritin assay is designed to have a mean recovery of 100 ± 10% when analyzing samples spiked with known amounts of ferritin. Known concentrations of ferritin were added to five human serum specimens. In a study, the concentration of ferritin was determined using the ARCHITECT Ferritin assay and the resulting percent recovery was calculated.

Sample	Endogenous Ferritin Conc. (ng/mL)	Ferritin Added (ng/mL)	Observed Ferritin Conc. (ng/mL)	Percent Recovery
1	21.19	85.72	100.27	92.3
	21.19	262.26	267.77	94.0
	21.19	423.92	440.40	98.9
2	82.17	85.72	171.85	104.6
	82.17	262.26	328.15	93.8
	82.17	423.92	510.35	101.0
3	175.40	85.72	250.67	87.8
	175.40	262.26	404.20	87.2
	175.40	423.92	606.72	101.7
4	246.95	85.72	327.98	94.5
	246.95	262.26	485.28	90.9
	246.95	423.92	662.04	97.9
5	452.76	85.72	550.16	113.6
	452.76	262.26	738.96	109.1
	452.76	423.92	924.84	111.4

Average Recovery: 98.6%

$$\% \text{ Recovery} = \frac{\text{Observed Ferritin Conc. (ng/mL)} - \text{Endogenous Ferritin Conc. (ng/mL)}}{\text{Ferritin Added (ng/mL)}} \times 100$$

Analytical Sensitivity

The ARCHITECT Ferritin assay is designed to have an analytical sensitivity of ≤ 1 ng/mL. Analytical sensitivity is defined as the concentration at two standard deviations from the mean RLU value of the ARCHITECT Ferritin MasterCheck Level 0 (0.0 ng/mL), and represents the lowest measurable concentration of ferritin that can be distinguished from zero. The mean analytical sensitivity of the ARCHITECT Ferritin assay was calculated to be < 1 ng/mL (n=36 runs).

Interference/Specificity

The ARCHITECT Ferritin assay is designed to have ≤ 10% mean interference from hemoglobin, bilirubin, triglycerides, and protein at the levels indicated below. Potential interference from these four components was studied in the ARCHITECT Ferritin assay. The ARCHITECT Ferritin assay demonstrated ≤ 10% mean interference at the levels indicated below.

- Hemoglobin 200 mg/dL
- Bilirubin 20 mg/dL
- Triglycerides 3000 mg/dL
- Protein 2 g/dL and 12 g/dL

Accuracy by Correlation

The ARCHITECT Ferritin assay demonstrated a slope of 1.2 ± 0.2 and a correlation coefficient (r) of ≥ 0.95 in the 1-1000 ng/mL range when compared to the AxSYM Ferritin assay. A study was performed where specimens were tested using ARCHITECT Ferritin assay and AxSYM Ferritin assay. Data from this study were analyzed using least squares and Passing Bablok regression methods and are summarized in the following table.***

Abbott ARCHITECT Ferritin vs. Abbott AxSYM Ferritin

Method	Number of Specimens	Range* (ng/mL)	Intercept	Slope	Correlation Coefficient
Least Squares	436	1 - 1000[1]	-4.86	1.18	0.979
Linear Regression	518	1 - 2000[2]	-3.65	1.18	0.986
Passing-Bablok**	436	1 - 1000[1]	-1.52	1.16	0.979
Linear Regression	518	1 - 2000[2]	-1.89	1.17	0.986

In this evaluation, serum specimens tested on the ARCHITECT Ferritin assay ranged from [1]1.39 to 1510.64 and [2]1.39 to 1967.46 ng/mL. Serum specimens tested on the AxSYM Ferritin assay ranged from [1]1.37 to 997.17 and [2]1.37 to 1827.47 ng/mL.

* Range established from AxSYM Ferritin values.

** A linear regression method with no special assumptions regarding the distribution of the samples and measurement errors.[23]

*** Representative data; results in individual laboratories may vary from these data.

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Folate protocol

ARCHITECT SYSTEM

en

Folate

REF 1P74

34-5955/R03

B1P740

Read Highlighted Changes
Revised April 2010

Folate

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REACTION VESSELS	Reaction Vessels	ASSAY SPECIFIC DILUENT	Assay Specific Diluent
SAMPLE CUPS	Sample Cups	PRE-TREATMENT REAGENT 1	Pre-Treatment Reagent (1, 2)
REPLACEMENT CAPS	Replacement Caps	SPECIMEN DILUENT	Specimen Diluent
REAGENT LOT	Reagent Lot	MANUAL DILUENT	Manual Diluent
CONTROL NO.	Control Number	RBC LYSIS DILUENT	Red Blood Cells Lysis Diluent
SEPTUM	Septum	LYSIS REAGENT	Lysis Reagent
ASSAY CD-ROM	Assay CD-ROM	PRE-TRIGGER SOLUTION	Pre-Trigger Solution
MICROPARTICLES	Microparticles	TRIGGER SOLUTION	Trigger Solution
CONJUGATE	Conjugate	WASH BUFFER	Wash Buffer

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

WARNING:
Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies. These specimens should not be assayed with the ARCHITECT Folate assay. Refer to the **LIMITATIONS OF THE PROCEDURE** section in this package insert.

NAME
ARCHITECT Folate

INTENDED USE
The ARCHITECT Folate assay is a chemiluminescent microparticle Folate Binding Protein assay for the quantitative determination of folate in human serum, plasma, and red blood cells on the ARCHITECT *i* System.

SUMMARY AND EXPLANATION OF TEST
Folates are a class of vitamin compounds related to pteroylglutamic acid (PGA), which serve as cofactors in the enzymatic transfer of single carbon units in a variety of metabolic pathways.[1,2] Folate mediated one-carbon metabolism represents one of the most important biochemical reactions that occur in cells. Folates are necessary for nucleic acid and mitochondrial protein synthesis, amino acid metabolism, and other cellular processes that involve single carbon transfers. Folates can serve as carbon donors or acceptors. Since different metabolic pathways require carbon groups with different levels of oxidation, cells contain numerous enzymes that change the oxidation state of carbon groups carried by folates[2] resulting in different metabolically active forms of folate. The predominant form of circulating folate is 5-methyltetrahydrofolic acid (5-mTHF). A methyl group is transferred from 5-mTHF to cobalamin in the pathway that links metabolism of folic acid and vitamin B12.[3]

Folate deficiency can be caused by low dietary intake, malabsorption due to gastrointestinal diseases, inadequate utilization due to enzyme deficiencies or folate antagonist therapy, drugs such as alcohol and oral contraceptives, and excessive folate demand, such as during pregnancy.[4] Because deficiencies of both vitamin B12 and folate can lead to megaloblastic (macrocytic) anemia, appropriate treatment requires differential diagnosis of the deficiency; thus, both vitamin B12 and folate values are needed. Low serum folate levels reflect the first stage of negative folate balance, and precede tissue depletion.[5] Low red-blood-cell folate values reflect the second stage of negative folate balance, and more closely correlate with tissue levels and megaloblastic anemia.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE
The ARCHITECT Folate assay is a two-step assay for the quantitative determination of folate in human serum, plasma, and red blood cells (RBC) using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Two pre-treatment steps mediate the release of folate from endogenous folate binding protein. In Pre-Treatment Step 1, sample and Pre-Treatment Reagent 2 (Dithiothreitol or DTT) are aspirated and dispensed into a reaction vessel (RV). In Pre-Treatment Step 2, an aliquot of sample/Pre-Treatment Reagent 2 mixture is aspirated and dispensed into a second RV. Pre-Treatment Reagent 1 (potassium hydroxide or KOH) is then added. An aliquot of the pre-treated sample is transferred into a third RV, followed by the addition of Folate Binding Protein (FBP) coated paramagnetic microparticles and assay specific diluent. Folate present in the sample binds to the FBP coated microparticles. After washing, pteroic acid-acridinium labeled conjugate is added and binds to unoccupied sites on the FBP-coated microparticles. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of folate in the sample and the RLUs detected by the ARCHITECT *i* optical system.

In the Folate RBC assay, an initial manual pre-treatment step converts RBC-bound folate to measurable folate, after which these samples are processed as described above.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS
Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT Folate Reagent Kit (1P74-25, 1P74-35)

- **MICROPARTICLES** 1 Bottle (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) Anti-Folate Binding Protein (mouse, monoclonal) coupled to microparticles affinity-bound with Folate Binding Protein (bovine), in TRIS buffer with protein stabilizers (human serum albumin and caprine). Minimum concentration: 0.08% solids. Preservatives: sodium azide and antimicrobial agents.
- **CONJUGATE** 1 Bottle (29.0 mL per 100-test bottle/29.0 mL per 500-test bottle) Pteroic Acid (PTA) - acridinium labeled conjugate in MES buffer with protein stabilizer (porcine). Minimum concentration: 4 ng/mL. Preservative: antimicrobial agents.
- **ASSAY SPECIFIC DILUENT** 1 Bottle (5.7 mL per 100-test bottle/25.3 mL per 500-test bottle) Folate Assay Specific Diluent containing borate buffer. Preservatives: sodium azide and antimicrobial agents.
- **PRE-TREATMENT REAGENT 1** 1 Bottle (50.2 mL per 100-test bottle/50.2 mL per 500-test bottle) Folate Pre-Treatment Reagent 1 containing potassium hydroxide.
- **PRE-TREATMENT REAGENT 2** 1 Bottle (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) Folate Pre-Treatment Reagent 2 containing dithiothreitol (DTT) in acetic acid buffer with EDTA.
- **SPECIMEN DILUENT** 1 Bottle (5.5 mL per 100-test bottle/25.9 mL per 500-test bottle) Folate Specimen Diluent containing TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

Manual Diluent

ARCHITECT Folate Manual Diluent (1P74-50)

- **MANUAL DILUENT** 1 Bottle (4 mL) Folate Manual Diluent containing TRIS buffer with protein stabilizer (human serum albumin). Preservative: sodium azide.

Other Reagents

ARCHITECT Folate RBC Lysis Diluent (1P74-40)

- **RBC LYSIS DILUENT** 1 Bottle (12.5 mL) Folate RBC Lysis Diluent (L2) containing citric acid and guanidine hydrochloride. Preservative: antimicrobial agent.

Folate Lysis Reagent (3P21-60)

- **LYSIS REAGENT** 4 Bottles (285-385 mg each) Folate Lysis Reagent (L1) containing ascorbic acid and guanidine hydrochloride.

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

NOTE: Bottle and volume varies based on order.

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservative: antimicrobial agents.

WARNINGS AND PRECAUTIONS
- **IVD**
- For *In Vitro* Diagnostic Use.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens[6]. Biosafety Level 2[7] or other appropriate biosafety practices[8,9] should be used for materials that contain or are suspected of containing infectious agents.
- The human serum albumin used in the Microparticles, Specimen Diluent, and Manual Diluent has been tested and found to be nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.

- Folate Pre-Treatment Reagent 1 contains potassium hydroxide (KOH) and is classified per applicable European Community (EC) Directives as: Corrosive (C). The following are the appropriate Risk (R) and Safety (S) phrases.

R34 Causes burns.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35 This material and its container must be disposed of in a safe way.
S36/37/39 Wear suitable protective clothing, gloves, and eye/face protection.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

- This product contains sodium azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.
- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagents, calibrators, or controls beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT Folate Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- **Prolonged exposure of Folate Pre-Treatment Reagent 1 to air without septum in place may compromise performance.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, and have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

NOTE: Hereafter, instructions in this package insert that pertain ONLY to the Folate RBC assay are contained in a text box.

-

The ARCHITECT Folate Reagent Kit, Folate RBC Lysis Diluent, Folate Manual Diluent, and the Controls must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.

NOTE: The ARCHITECT Folate Reagent Kit is shipped cold and should be stored at 2-8°C after receipt. Calibrators are shipped frozen and must be stored at -10°C or colder.

- Calibrators and Controls are sensitive to light. **Store bottles in carton to protect from light.**

- Unreconstituted Folate Lysis Reagent (L1) must be stored at 15-30°C. Reconstituted Folate Lysis Reagent (L1) must be stored at 2-8°C. The expiration date is 7 days from the date of reconstitution. Write the expiration date of the reconstituted Folate Lysis Reagent (L1) on the bottle but do not exceed the lot expiration date printed on the bottle.

- The ARCHITECT Folate Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT Folate (1P74) assay files are named "Folate II" and "FolateRBC".
- The ARCHITECT Folate II (assay number 685) and/or FolateRBC (assay number 686) assay file(s) must be installed on the ARCHITECT *i* System before performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be installed on the ARCHITECT *i* System prior to performing the assay. For information on installing and deleting maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 2.
- ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be run at a minimum once every 24 hours. For laboratories processing a higher volume of B12 (List 6C09) and Folate tests on a single module, this procedure must be run more than once in a 24-hour period.
  - If B12 (List 6C09) and Folate are run on a single module and you run > 100 B12 (List 6C09) or > 100 Folate tests in 24 hours, perform the *6041 Daily Maintenance* procedure (version 5 or higher) after every 100 B12 (List 6C09) or 100 Folate tests run.
  - Refer to **LIMITATIONS OF THE PROCEDURE** for additional information.
- If microbial contamination is suspected when running ARCHITECT Folate on the ARCHITECT *i* System due to shifts in results and/or the incidence of calibration failures with the following error codes:
  - 1402 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, calibrators incorrectly loaded
  - 1206 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, concentration too high for Cal A
  - 1120 - Assay (Folate II/FolateRBC). Number (685/686) Calibration failure, fit response too low for Cal A

  the following actions must be taken to protect the integrity of assay results:
  - Contact your local customer support representative to schedule the local Abbott Service Representative to perform the *2180 Internal Decontamination* procedure on your ARCHITECT *i* System. If the instrument is connected to an Automatic Reconstitution Module (ARM), the *2182 ARM Decontamination* procedure must also be executed.
  - It may be necessary to repeat the decontamination procedure if microbial contamination recurs.

- When configuring the host for the Folate RBC assay, set the appropriate default dilutions:
  - If running whole blood specimens or whole blood controls, configure the default dilution as "RBC DIL".
  - If running controls other than whole blood controls, configure the default dilution as "UNDILUTED".

- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

- The default result unit for the ARCHITECT Folate assay is ng/mL. An alternate result unit, nmol/L, may be selected for reporting results by editing assay parameter "Result concentration units", to nmol/L. The conversion factor used by the ARCHITECT *i* System is 2.265.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen collection tubes listed below were verified for use with the ARCHITECT Folate assays. Other specimen collection tubes have not been tested with these assays.

Folate	Folate RBC
• Serum (glass or plastic tube) • Serum separator (SST) • Lithium heparin plasma • Lithium heparin plasma separator (PST)	• Whole blood dipotassium EDTA (K_2 EDTA) • Whole blood tripotassium EDTA (K_3 EDTA)

- **Do not use human plasma collected in dipotassium or tripotassium EDTA tubes for Folate.**

- **Do not use human whole blood collected in lithium heparin tubes for Folate RBC.**

- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT Folate assays.
- Human serum, plasma, or whole blood specimens to be tested for folate should be protected from light.[10,11]
- Serum or plasma specimens should be collected from fasting individuals. Recent food intake may appreciably increase the folate concentration.[11]
- Do not use hemolyzed specimens. Serum or plasma specimens that are hemolyzed will give falsely elevated folate levels.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - hemolyzed
  - obvious microbial contamination
- Performance has not been established for the use of cadaveric specimens or body fluids other than human serum, plasma, and whole blood.
- For accurate results, serum or plasma specimens should be free of fibrin, red blood cells and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation. Serum or plasma specimens containing red blood cells may give falsely elevated folate levels.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged before testing if
  - they contain fibrin, red blood cells, or other particulate matter, or
  - they were frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.

Storage

- Human serum, plasma, or whole blood specimens to be tested for folate should be protected from light.[10,11]
- Remove serum from clot or separator gel as soon as possible after complete clot formation. If testing will not be performed immediately, serum specimens may be stored either at 2-8°C for up to 7 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Remove plasma from red blood cells as soon as possible upon receipt.[11] If testing will not be performed immediately, plasma specimens may be stored either at 2-8°C for up to 7 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Avoid more than 3 freeze/thaw cycles.

- For red blood cell folate measurements, mix whole blood tube by inverting 10 times to ensure a homogeneous sample. **Determine the hematocrit of each specimen prior to storage.** The hematocrit value will be required in Calculations 1 and 2 beginning on page 6.
- If testing will not be performed immediately, whole blood specimens may be stored at 2-8°C for up to 2 days or frozen (-10°C or colder) for up to 30 days prior to being tested.
- Avoid more than 1 freeze/thaw cycle.

Shipping

- Before shipping specimens, it is recommended that serum and plasma specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens must be shipped frozen (-10°C or colder) on dry ice and protected from light. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 1P74 ARCHITECT Folate Reagent Kit

Materials Required but not Provided

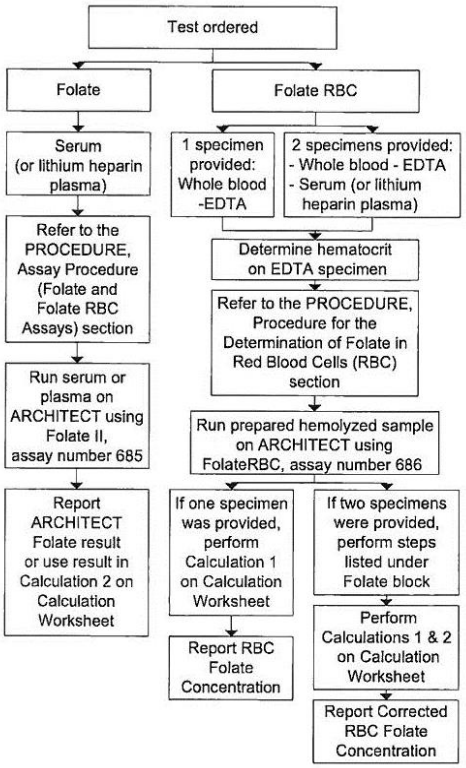
- ARCHITECT *i* System
- ARCHITECT *i* **ASSAY CD-ROM**
- 1P74-01 ARCHITECT Folate Calibrators
- 1P74-10 ARCHITECT Folate Controls
- 1P74-50 ARCHITECT Folate **MANUAL DILUENT**
- 1P74-40 ARCHITECT Folate **RBC LYSIS DILUENT**
- 3P21-60 Folate **LYSIS REAGENT**
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure Overview

The Folate result is obtained using serum or plasma specimens. The Folate RBC result is obtained using a hemolysate prepared from whole blood. The Folate RBC result includes folate present in the RBCs and in the plasma. In order to obtain the folate concentration only in the RBCs, both specimens are required and a calculation is performed using results from both assays to obtain a Corrected RBC Folate result (if desired). The three paths are shown in the flowchart below based on the specimens provided.

NOTE: The ARCHITECT Folate (1P74) assay files are named "Folate II" and "FolateRBC".



Procedure for the Determination of Folate in Red Blood Cells (RBC)

NOTE: Determine hematocrit of EDTA specimen. This value will be required in Calculations 1 and 2 beginning on page 6.

Part 1: Reconstitution of Folate Lysis Reagent (L1)

- Reconstitute one bottle of the Folate Lysis Reagent (L1) by adding 30 mL distilled or deionized water.
- Cap the reagent bottle and mix by inverting 10 times and let stand for 15 minutes.
- The expiration date is 7 days from the date of reconstitution. Write the expiration date of the reconstituted Folate Lysis Reagent (L1) on the line provided on the bottle label, but do not exceed the lot expiration date printed on the bottle. Store at 2-8°C when not in use.

Part 2: Preparation of Red Blood Cell Hemolysate

NOTE: The assay must be initiated on the final hemolysate within 2 hours.

- Invert the reconstituted Folate Lysis Reagent (L1) an additional 10 times. Pipette 1.0 mL into a suitable sample tube with a cap (example: 2 mL tube).
- Mix whole blood tube by inverting 10 times to ensure a homogeneous sample.
- Add 100 µL of whole blood sample to the sample tube containing the 1.0 mL of the reconstituted Folate Lysis Reagent (L1).
- Cap the tube and mix by inverting 10 times or vortexing and allow to stand at room temperature (15-30°C) for 90 minutes (± 5 minutes). **Protect from light.**
- Pipette 100 µL ARCHITECT Folate RBC Lysis Diluent (L2) into a new sample tube (or ARCHITECT sample cup). Then add 100 µL of hemolyzed sample.
- Mix by swirling or vortexing and initiate assay on this sample within 2 hours.

Assay Procedure (Folate and Folate RBC Assays)

- Before loading the ARCHITECT Folate Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Place a septum on the bottle. For instructions about placing septums on bottles, refer to the Handling Precautions section of this package insert.
- Load the ARCHITECT Folate Reagent Kit on the ARCHITECT *i* System.
  - Verify that all the necessary assay reagents are present.
  - Ensure that septums are present on all reagent bottles. Refer to ARCHITECT Operations Manual, Section 5, for details on how to load reagents.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.

  NOTE: The ARCHITECT Folate (1P74) assay files are named "Folate II" and "FolateRBC".
- Select the appropriate assay protocol.
  - If running a serum or plasma specimen/control, select Folate II (assay number 685, "UNDILUTED").
  - If running an automated dilution on a serum or plasma specimen, select the 1:2 protocol of Folate II (assay number 685, "1:2").
  - If running a whole blood specimen or whole blood control, select FolateRBC (assay number 686, "RBC DIL").
  - If running controls other than whole blood controls with the FolateRBC assay, select the undiluted protocol of FolateRBC (assay number 686, "UNDILUTED").
  - For additional information on ordering patient specimens, calibrators, and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Order list report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

- Priority: 85 µL for the first ARCHITECT Folate test plus 35 µL for each additional ARCHITECT Folate test from the same sample cup.
- ≤ 3 hours on board: 150 µL for the first ARCHITECT Folate test plus 35 µL for each additional ARCHITECT Folate test from the same sample cup.
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

- Prepare calibrators and controls.
  - Mix the ARCHITECT Folate Calibrators and Controls by gentle inversion (3-5 times) prior to use.
  - Discard ARCHITECT Folate Calibrators after 3 freeze/thaw cycles.
  - To obtain the recommended volume requirements for the ARCHITECT Folate Calibrators and Controls, hold the bottles vertically, and dispense 6 drops of each calibrator or 6 drops of each control into each respective sample cup.

NOTE: It is very important to return the ARCHITECT Folate Calibrators and Controls to their carton and correct storage conditions immediately after use, as follows.
- **Store ARCHITECT Folate Calibrators at -10°C or colder.**
- **Store ARCHITECT Folate Controls at 2-8°C.**

  - Load samples. For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN. For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures (for folate serum or plasma determinations only)

Specimens with a folate serum or plasma value exceeding 20.0 ng/mL are flagged with the code " > 20.0" and may be diluted using either the Automated Dilution Procedure or the Manual Dilution Procedure.

Automated Dilution Procedure

- If using the Automated Dilution Protocol (assay number 685, 1:2 Protocol), the system performs a 1:2 dilution. The system will use the dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.

Manual Dilution Procedure

- The suggested dilution for ARCHITECT Folate is 1:2. It is recommended dilutions not exceed 1:4.
- For a 1:2 dilution, add 100 µL of the patient specimen to 100 µL of ARCHITECT Folate Manual Diluent (1P74-50). For a 1:4 dilution, add 100 µL of the patient specimen to 300 µL of ARCHITECT Folate Manual Diluent (1P74-50).
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Separate calibrations are required for ARCHITECT Folate II and ARCHITECT FolateRBC assay files.
- To perform a calibration, test ARCHITECT Calibrators A through F in duplicate. A single sample of all levels of ARCHITECT Folate Controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- Calibration Range: 0.0 - 20.0 ng/mL.
- Once a calibration is accepted and stored, all subsequent samples may be tested off the appropriate calibration curve without further calibration unless one or more of the following occur:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For best results, establish statistically-based QC ranges to monitor and control the frequency of recalibration.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Folate assay is that a single sample of each control be tested once every 24 hours each day of use. If your laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated test results are invalid and samples must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Folate assay belongs to method group 1.

RESULTS

The ARCHITECT Folate assay uses a 4 Parameter Logistic Curve fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Alternate Result Units

- The default result unit for the ARCHITECT Folate assay is ng/mL. When the alternate result unit, nmol/L, is selected, the conversion factor used by the system is 2.265.
- Conversion Formula: (concentration in ng/mL) x (2.265) = nmol/L
- Formulas and examples indicate ng/mL as the result unit. If the chosen ARCHITECT Folate result is nmol/L, the final result would be in nmol/L.

Calculation of Red Blood Cell Folate Concentration (for Folate RBC Assay only):

Calculation performed by the ARCHITECT *i* System

When the FolateRBC assay is used (assay number 686 utilizing "RBC DIL" protocol), the ARCHITECT *i* System automatically corrects the reported sample result for dilutions that were required during the preparation of the red blood cell hemolysate. This is the ARCHITECT FolateRBC test result. **Do not report this result. Further calculation is required.**

NOTE: A Calculation Worksheet is provided at the end of this package insert to assist with RBC Folate calculations.

Calculations performed by the Operator

Calculation 1

To calculate the RBC Folate concentration from the ARCHITECT FolateRBC test result, use the following formula:

$$\text{RBC Folate Concentration (ng/mL)} = \frac{A}{B} \times 100$$

where:

A = ARCHITECT FolateRBC test result (ng/mL)

B = % Hematocrit (value obtained prior to storage or prior to Procedure for Folate RBC)

Example:

ARCHITECT FolateRBC test result = 64.0 ng/mL

% Hematocrit = 32

$$\text{RBC Folate Conc.} = \frac{64.0 \text{ ng/mL}}{32} \times 100 = 200.0 \text{ ng/mL}$$

Tube Type Matrix Comparison

The following tube types are acceptable for use with the ARCHITECT Folate assay:

- Glass: Serum
- Plastic: Serum, Serum Separator Tube (SST), Lithium Heparin Plasma Tube, and Lithium Heparin Plasma Separator Tube (PST),

On average, the tube types evaluated showed less than a 10% difference when compared to the control tube type (plastic serum). The distribution of the percent differences per tube type is listed in the following table.

Evaluation Tube Type	Distribution of Absolute %Differences[a]	
	< 10%	10% to 20%
Glass, Serum	92.6% (25/27)	7.4% (2/27)
Serum Separator Tube, Plastic (SST)	100.0% (27/27)	0.0% (0/27)
Lithium Heparin Plasma Tube	80.0% (20/25)	20.0% (5/25)
Lithium Heparin Plasma Separator Tube (PST)	92.6% (25/27)	7.4% (2/27)

[a] There were no absolute % difference values > 20%.

The following tube types are acceptable for use with the ARCHITECT Folate RBC assay:

- whole blood dipotassium EDTA (K_2 EDTA)
- whole blood tripotassium EDTA (K_3 EDTA)

All K3 EDTA tubes evaluated (n=27) showed less than 10% difference when compared to matched K2 EDTA tubes.

Method Comparison

Two correlation studies were performed based on guidance from CLSI document EP9-A2[27] using the Passing-Bablok[28] regression method to compare the ARCHITECT Folate assay to the AxSYM Folate assay. One study was performed with serum/plasma specimens and the other with whole blood specimens. The analysis of the results from the serum/plasma study included both the full range of specimens analyzed and a truncated range for the AxSYM Folate assay. The truncated range minimizes any effects due to apparent non-linearity of AxSYM results at the clinically less significant higher folate concentrations. Truncation was unnecessary for whole blood specimens. The tables below summarize the results of these correlation analyses.

Correlation of ARCHITECT Folate to AxSYM Folate

Specimen Type	Conc. Range ng/mL (nmol/L)		r[a]	Intercept ng/mL (nmol/L)	Slope
	ARCHITECT	AxSYM			
Serum/Plasma (n=144)	0.9-28.9 (2.0-65.5)	2.2-33.4 (5.0-75.7)	0.921	-5.85 (-13.25)	1.27
Truncated Serum/Plasma (n=43)	0.9-12.6 (2.0-28.5)	2.2-14.0 (5.0-31.7)	0.963	-1.19 (-2.70)	0.82
Whole Blood (n=123)	145.5-1014.6 (329.6-2298.1)	155.8-1034.5 (352.9-2343.1)	0.895	-33.36 (-75.56)	0.74

[a] r = Correlation Coefficient

Some serum and plasma samples in the upper region of the dynamic range may read lower in the AxSYM Folate assay when compared to the ARCHITECT Folate assay. This can result in a decreased correlation coefficient value over the entire measurement range.

Two correlation studies were performed using the Passing-Bablok regression method to compare the ARCHITECT Folate assay to the ARCHITECT Folate Non-US assay. One study was performed with serum/plasma specimens and the other with whole blood specimens. The table below summarizes the results of these correlation studies.

Correlation of ARCHITECT Folate to ARCHITECT Folate Non-US

Specimen Type	Conc. Range ng/mL (nmol/L)		r[a]	Intercept ng/mL (nmol/L)	Slope
	ARCHITECT	ARCHITECT Non-US			
Serum/Plasma (n=140)	1.4-28.9 (3.2-65.5)	1.0-31.3 (2.3-70.9)	0.997	-0.38 (-0.86)	0.98
Whole Blood (n=131)	145.5-1014.6 (329.6-2298.1)	97.8-900.5 (221.5-2039.6)	0.973	55.67 (126.09)	0.89

[a] r = Correlation Coefficient

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The following US Patents are relevant to the ARCHITECT *i* System or its components. There are other such patents and patent applications in the United States and worldwide.

5 468 646	5 543 524	5 545 739
5 565 570	5 669 819	5 783 699

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Calculation 2
Calculation of Corrected Red Blood Cell Folate Concentration (for Folate RBC Assay only):

Folate concentrations from serum or plasma are very small as compared to RBC folate concentrations, in most cases. It is possible for the serum or plasma folate concentration to be within or above the expected normal range while the RBC folate concentration is below the expected normal range. In these instances, a correction may be needed. The Folate serum (or plasma) result is required for this calculation. The following calculation will correct for serum or plasma folate concentrations:

$$\text{Corrected RBC Folate Conc. (ng/mL)} = C - \left[D \times \left[\frac{100 - B}{B} \right] \right]$$

where:

B = % Hematocrit (value used for B in Calculation 1)

C = RBC Folate Concentration result from Calculation 1 (ng/mL)

D = ARCHITECT Folate Serum (or plasma) result (ng/mL)

Example:

% Hematocrit = 32

RBC Folate Concentration result = 200 ng/mL

ARCHITECT Folate Serum (or plasma) result = 25.0 ng/mL

Corrected RBC Folate Conc.=

$$200.0 \text{ ng/mL} - \left[25.0 \text{ ng/mL} \times \left[\frac{100 - 32}{32} \right] \right] = 146.9 \text{ ng/mL}$$

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measuring Interval

The measuring interval of the ARCHITECT Folate assay is 1.5 ng/mL to 20.0 ng/mL.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes the ARCHITECT Folate assay result should be used in conjunction with other data, *e.g.*, other clinical testing, symptoms, clinical impressions, etc.
- If the folate level is inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies.[12,13]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[14] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
- Serum or plasma containing red blood cells may give falsely elevated folate levels. These samples should be centrifuged prior to use. Serum or plasma samples that are hemolyzed will give falsely elevated folate levels.
- Serum and plasma specimens from patients with renal impairment or failure (including dialysis patients) may exhibit varying degrees of falsely depressed folate values.[15] Therefore, to evaluate folate patients with renal impairment or failure, it is recommended that low ARCHITECT Folate values be confirmed by an alternate folate method such as the ARCHITECT Folate RBC assay.
- Methotrexate, aminopterin, and folinic acid (Leucovorin) are chemotherapeutic agents whose molecular structures are similar to folate. These agents cross react with folate binding protein in folate assays.[16]
- Samples to be tested for folate should be protected from light. Light accelerates the degradation of folate.
- Accumulation of denatured protein from the pre-treatment step in the sample probe may impact results of other assays on the ARCHITECT *i* System. ARCHITECT maintenance procedure *6041 Daily Maintenance* (version 5 or higher) must be run to eliminate this effect. Refer to the **INSTRUMENT PROCEDURE** section for instructions.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal and deficient ranges, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) document C28-A3.[17] The nutritional status of the specimen donors was unknown. All specimens tested were from fasting, apparently healthy males and non-pregnant females greater than 18 years old from a UK population. Serum and whole blood samples were tested for serum/plasma and red blood cell folate using the ARCHITECT Folate assay. Data from this study are summarized in the following table.

	Expected Values Data Statistics ng/mL (nmol/L)			
	n	Min	Max	Expected Values
Serum/Plasma	155	1.6 (3.6)	19.5 (44.2)	3.1 - 20.5 (7.0 - 46.4)
Whole Blood	168	58.5 (132.5)	733.1 (1660.5)	126.0 - 651.1 (285.4 - 1474.7)

Folate Deficients/Indeterminates

- Folate deficiency is typically associated with serum levels less than 3.5 ng/mL or RBC levels less than 150 ng/mL.[18,21]
- Patients with RBC folate levels ranging from 150 to 250 ng/mL have been associated with megaloblastic erythropoiesis, but folate values in patients with normal erythropoiesis can also fall within this range.[18]
- Often, the diagnosis of folate deficiency cannot be based solely on serum or RBC folate levels, and further testing may be required.[18-20]

SPECIFIC PERFORMANCE CHARACTERISTICS

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT Folate assay is designed to have a within-laboratory imprecision of:

- ≤ 12% total CV for serum samples from 3.5 ng/mL to 20 ng/mL and ≤ 11% CV for RBC hemolysate between 150 ng/mL and 640 ng/mL.
- a Standard Deviation (SD) ≤ 0.42 for serum samples below 3.5 ng/mL and SD ≤ 16.50 for RBC hemolysate samples below 150 ng/mL.

A study was performed based on guidance from the CLSI document EP5-A2.[22] Three serum panels (S1, S2, and S3) and three hemolysate panels (H1, H2, and H3) were assayed, using 1 instrument, in replicates of 3, at two separate times per day for 20 days, using 2 lots of reagents. Data from this study are summarized in the following table.

ARCHITECT Folate Within-Laboratory Precision

Sample Level	Reagent Lot	Mean ng/mL (nmol/L)	Within-Run SD ng/mL (nmol/L)	Within-Run %CV	Total SD ng/mL (nmol/L)	Total %CV
S1	1	3.5 (7.9)	0.12 (0.27)	3.5	0.14 (0.32)	3.9
	2	3.6 (8.2)	0.14 (0.32)	3.9	0.17 (0.39)	4.7
S2	1	10.8 (24.5)	0.18 (0.41)	1.7	0.41 (0.93)	3.8
	2	11.2 (25.4)	0.21 (0.48)	1.9	0.44 (1.00)	4.0
S3	1	16.8 (38.1)	0.27 (0.61)	1.6	0.53 (1.20)	3.1
	2	17.0 (38.5)	0.24 (0.54)	1.4	0.61 (1.38)	3.6
H1	1	113.2 (256.4)	6.10 (13.82)	5.4	8.82 (19.98)	7.8
	2	118.1 (267.5)	4.69 (10.62)	4.0	6.49 (14.70)	5.5
H2	1	222.9 (504.9)	7.04 (15.95)	3.2	13.29 (30.10)	6.0
	2	221.9 (502.6)	5.59 (12.66)	2.5	12.19 (27.61)	5.5
H3	1	367.2 (831.7)	7.87 (17.83)	2.1	21.60 (48.92)	5.9
	2	359.1 (813.4)	8.90 (20.16)	2.5	22.97 (52.03)	6.4

Autodilution Verification

The ARCHITECT Folate assay was designed to have an absolute mean change in measured concentration of ≤ 20% when comparing manual to autodilution (1:2 dilution). The assay was evaluated for autodilution with the 1:2 autodilution method vs. the 1:2 and 1:4 manual dilution methods using 18 specimens with folate values ranging from 20 to 40 ng/mL. Three replicates each of the autodiluted and manually diluted samples were assayed on one instrument using the ARCHITECT Folate assay. Data from this study are summarized in the following table.

Percent Differences Across Samples

Dilution Comparison	n	Mean/Median %Difference
Auto (1:2) vs. Manual (1:2)	18	0.8
Auto (1:2) vs. Manual (1:4)	18	5.9

Linearity

The ARCHITECT Folate assay was evaluated for linearity by mixing a high (> 20 ng/mL) serum specimen pool in specific ratios with a low (≤ 3.5 ng/mL) serum specimen pool to create 11 mixed sample pools. All pools were tested by the ARCHITECT Folate assay. Based on guidance from CLSI document EP6-A[23], the study demonstrated linearity from 1.6 to 20 ng/mL.

Accuracy to World Health Organization (WHO) Standard

The ARCHITECT Folate assay was evaluated for bias relative to the Folate WHO International Standard 03/178. A minimum of 38 replicates of the WHO Standard was tested on each of 2 instruments. A different reagent lot was used on each instrument and one calibrator lot was used for both instruments.

The Folate assay results were accurate within ± 10% to the 1st International Reference Standard (I.S.) for Serum Folate (03/178). Data from this study are summarized in the following table.

n	Median ng/mL (nmol/L)	Target ng/mL (nmol/L)	Diff.[a] ng/mL (nmol/L)	Two-Sided 95%CL[b] ng/mL (nmol/L)	%Diff.[a]	Two-Sided 95%CL[b] %Diff.[a]
76	5.4 (12.2)	5.3 (12.0)	0.1 (0.2)	0.0, 0.1 (0.0, 0.2)	1.3	-0.6, 1.3

[a] Diff. = Difference
[b] CL = Confidence Limit

Sensitivity

Sensitivity is defined as the Limit of Quantitation (LoQ), which is the lowest amount of analyte in a sample that can be accurately quantitated with a Total Error of ± 39%.[24]

The ARCHITECT Folate assay is designed to have an LoQ of ≤ 3.5 ng/mL.

The Limit of Blank (LoB), Limit of Detection (LoD), and LoQ of the ARCHITECT Folate assay were determined based on guidance from CLSI document EP-17A[25], using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using 1 zero-level (3 replicates) and 5 low-level folate samples (3 replicates each). The following values were determined in this study: LoB = 0.3 ng/mL (0.7 nmol/L), LoD = 0.5 ng/mL (1.1 nmol/L), and LoQ = 1.5 ng/mL (3.4 nmol/L).

Specificity

The specificity of the ARCHITECT Folate assay was evaluated by testing cross-reactivity with aminopterin, folinic acid, and methotrexate in processed human serum containing endogenous folate. Therapeutic levels of these drugs can greatly exceed the levels tested in this study and are expected to interfere with the ARCHITECT Folate assay.[16] A study was performed with the ARCHITECT Folate assay based on guidance from the CLSI document EP7-A2.[26] Aliquots of human serum at two different folate concentrations were supplemented with potential cross-reactants and tested for folate. Data from this study are summarized in the following table.

Interferent	Reference n	Reference Mean/ Median ng/mL	Test n	Test Mean/ Median ng/mL	Diff.[a] ng/mL	%Diff.[b]	%Cross-Reactivity[c]
Aminopterin	40	2.6	40	8.3	5.7	219.2	1.1
≥ 500 ng/mL	40	7.4	39	13.0	5.6	75.7	1.1
Folinic Acid	40	2.9	40	3.4	0.5	17.2	0.5
≥ 100 ng/mL	40	7.9	44	7.3	-0.6	-7.4	-0.6
Methotrexate	45	2.7	40	4.8	2.1	77.8	2.1
≥ 100 ng/mL	40	7.6	40	8.9	1.4	18.2	1.4

[a] Difference = test mean [or median] conc. - reference mean [or median] conc.
[b] % Difference = Difference / reference mean [or median] conc. x 100
[c] % Cross-Reactivity = Difference / interferent conc. x 100

Interference

Potential interference in the ARCHITECT Folate assay from bilirubin, (conjugated and unconjugated), triglycerides, and protein was demonstrated in a study based on guidance from CLSI document EP7-A2.[26] Hemoglobin was not tested due to the high folate content in red blood cells. Refer to the **LIMITATIONS OF THE PROCEDURE** section. Data from this study are summarized in the following table.

Interferent	Reference n	Reference Mean/ Median ng/mL	Test n	Test Mean/ Median ng/mL	Diff.[a] ng/mL	%Diff.[b]
Bilirubin (unconjugated)	40	2.1	40	2.0	-0.1	-4.0
≤ 20 mg/dL	40	7.9	40	7.6	-0.3	-3.8
Bilirubin (conjugated)	40	1.8	40	1.7	-0.1	-5.6
≤ 20 mg/dL	40	7.5	40	7.0	-0.5	-6.7
Protein	40	2.6	40	2.9	0.3	11.5
≤ 12 g/dL	40	8.8	40	9.1	0.3	2.8
Triglycerides	40	2.1	40	2.2	0.1	4.8
≤ 3000 mg/dL	40	7.9	39	8.0	0.1	1.8

[a] Difference = test mean [or median] - reference mean [or median]
[b] % Difference = Difference / reference mean [or median] x 100

Calculation Worksheet
(for RBC Folate Calculations)

Sample ID_____
Date_____
Initials_____

Notes: **The ARCHITECT Folate (1P74) assay files are named: "Folate II" and "FolateRBC".**
If only a whole blood specimen was provided for the Folate RBC test, perform Calculation 1.
If the Corrected RBC Folate Concentration result is desired and both a whole blood specimen and a serum or plasma specimen were provided, perform Calculations 1 and 2.
(See flowchart in Assay Procedure Overview section.)

Calculation 1
Calculate the RBC Folate Concentration.

Step 1. ***Record values.***

A = ARCHITECT FolateRBC test result (ng/mL) (value reported by ARCHITECT) _____ (A)

B = % Hematocrit (value obtained in the Storage section on page 4) _____ (B)

Step 2. ***Perform calculation.***

$$\text{RBC Folate Concentration (ng/mL)} = \frac{A}{B} \times 100 = \text{\_\_\_\_\_ (C)}$$

This is the RBC Folate Concentration result. (C)

If the Corrected RBC Folate Concentration result is desired, perform Calculation 2 to correct for the serum (or plasma) Folate concentration. (The Folate serum (or plasma) result is required for this calculation.)

Calculation 2
Calculate the Corrected RBC Folate Concentration.

Step 1. ***Record values.***

B = % Hematocrit (value used for B in Calculation 1) _____ (B)

C = RBC Folate Concentration result from Calculation 1 (ng/mL) _____ (C)

D = ARCHITECT Folate Serum (or plasma) test result (ng/mL) _____ (D)

Step 2. ***Perform calculation by following the steps listed below the equation.***

$$\text{Corrected RBC Folate Conc. (ng/mL)} = C - \left[D \times \left[\frac{100 - B}{B} \right] \right]$$

Subtract B from 100. _____ (E)

Divide result obtained in (E) by B. _____ (F)

Multiply result otained in (F) by D. _____ (G)

Subtract (G) from C. _____ (H)

This is the Corrected RBC Folate Concentration result (H).

Vitamin B12 Protocol

ARCHITECT SYSTEM

en
B12
REF 7K61
B7K610
G3-0916/R08

Read Highlighted Changes
Revised February 2013

B12

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	CONTROL L	Control Low, Medium, High (L, M, H)
IVD	*In Vitro* Diagnostic Medical Device	REACTION VESSELS	Reaction Vessels
LOT	Lot Number	SAMPLE CUPS	Sample Cups
	Expiration Date	REPLACEMENT CAPS	Replacement Caps
8°C 2°C	Store at 2-8°C	REAGENT LOT	Reagent Lot
	Consult instructions for use	DANGER: REPRODUCTIVE HAZARD	Danger: Reproductive Hazard
CAL A	Calibrator A-F	WARNING: SENSITIZER	Warning: May cause an allergic reaction
SN	Serial Number	CONTAINS: AZIDE	Contains sodium azide. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number		
SEPTUM	Septum	WARNING: SEVERE IRRITANT	Warning: Severe Irritant
	Manufacturer	GTIN	Global Trade Item Number
		PRODUCT OF IRELAND	Product of Ireland

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

NAME

ARCHITECT B12

INTENDED USE

The ARCHITECT B12 assay is a Chemiluminescent Microparticle Intrinsic Factor assay for the quantitative determination of vitamin B12 in human serum and plasma on the ARCHITECT *i* System.

SUMMARY AND EXPLANATION OF TEST

Vitamin B12 (B12), a member of the corrin family, is a cofactor for the conversion of methylmalonyl Coenzyme-A (CoA) to succinoyl CoA. In addition, B12 is a cofactor in the synthesis of methionine from homocysteine, is implicated in the formation of myelin, and, along with folate, is required for DNA synthesis.[1,2]

B12 is absorbed from food after binding to a protein called intrinsic factor which is produced by the stomach. Causes of vitamin B12 deficiency can be divided into three classes: nutritional deficiency, malabsorption syndromes, and other gastrointestinal causes. B12 deficiency can cause megaloblastic anemia (MA), nerve damage and degeneration of the spinal cord. Lack of B12, even mild deficiencies, damages the myelin sheath that surrounds and protects nerves, which may lead to peripheral neuropathy. The nerve damage caused by a lack of B12 may become permanently debilitating, if the underlying condition is not treated. People with intrinsic factor defects who do not get treatment eventually develop a MA called pernicious anemia (PA).[2]

The relationship between B12 levels and MA is not always clear in that some patients with MA will have normal B12 levels; conversely, many individuals with B12 deficiency are not afflicted with MA. Despite these complications, however, in the presence of MA (e.g., elevated mean corpuscular volume (MCV)) there is usually serum B12 or folate deficiency.[2,3]

The true prevalence of B12 deficiency in the general population is unknown but increases with age. In one study,[4] fifteen percent of adults older than 65 years old had laboratory evidence of vitamin B12 deficiency.

A serum B12 level below the normal expected range may indicate that tissue B12 levels are becoming depleted. However, a B12 level in the low normal range does not ensure that B12 levels are healthy and symptomatic patients should be further evaluated with tests for holotranscobalamin,[5] homocysteine and methylmalonic acid.[6,7]

There are a number of conditions that are associated with low serum B12 levels, including iron deficiency, normal near-term pregnancy, vegetarianism, partial gastrectomy/ileal damage, celiac disease, use of oral contraception, parasitic competition, pancreatic deficiency, treated epilepsy, and advancing age.[2,8-10,11] Disorders associated with elevated serum B12 levels include renal failure, liver disease, and myeloproliferative diseases.[8,12]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT B12 assay is a two-step assay with an automated sample pretreatment, for determining the presence of B12 in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex.

Sample and Pre-Treatment Reagent 1, Pre-Treatment Reagent 2, and Pre Treatment Reagent 3 are combined. An aliquot of the pre-treated sample is aspirated and transferred into a new Reaction Vessel (RV). The pre-treated sample, assay diluent, and intrinsic factor coated paramagnetic microparticles are combined. B12 present in the sample binds to the intrinsic factor coated microparticles. After washing, B12 acridinium labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of B12 in the sample and the RLUs detected by the ARCHITECT *i* optical system.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT B12 Reagent Kit (7K61)

- **MICROPARTICLES** 1 Bottle (6.6 mL per 100 test bottle / 27.0 mL per 500 test bottle) Intrinsic Factor (porcine) coated Microparticles in borate buffer with protein (bovine) stabilizers. Minimum Concentration: 0.1% solids. Preservative: antimicrobial agents.
- **CONJUGATE** 1 Bottle (5.9 mL per 100 test bottle / 26.3 mL per 500 test bottle) B12 acridinium-labeled Conjugate in MES buffer. Minimum concentration: 0.7 ng/mL. Preservative: ProClin.
- **ASSAY DILUENT** 1 Bottle (10.0 mL per 100 test bottle / 51.0 mL per 500 test bottle) B12 Assay Diluent containing borate buffer with EDTA. Preservative: antimicrobial agents.
- **PRE-TREATMENT REAGENT 1** 1 Bottle (27.0 mL per 100 test bottle / 50.4 mL per 500 test bottle) B12 Pre-Treatment Reagent 1 containing 1.0 N sodium hydroxide with 0.005% potassium cyanide.
- **PRE-TREATMENT REAGENT 2** 1 Bottle (5.5 mL per 100 test bottle / 25.9 mL per 500 test bottle) B12 Pre-Treatment Reagent 2 containing alpha monothioglycerol and EDTA.
- **PRE-TREATMENT REAGENT 3** 1 Bottle (5.5 mL per 100 test bottle / 25.9 mL per 500 test bottle) B12 Pre-Treatment Reagent 3 containing cobinamide dicyanide in borate buffer with protein (avian) stabilizers. Preservative: sodium azide.

Assay Diluent

ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50)

- **MULTI-ASSAY MANUAL DILUENT** 1 Bottle (100 mL) ARCHITECT *i* Multi-Assay Manual Diluent containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.[13] Biosafety Level 2[14] or other appropriate biosafety practices[15,16] should be used for materials that contain or are suspected of containing infectious agents.

The following warnings and precautions apply to these components:

- Microparticles
- Assay Diluent
- Pre-Treatment Reagent 3

DANGER:	Contains sodium borate.
H360	May damage fertility or the unborn child.
Prevention	
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P281	Use personal protective equipment as required.
Response	
P308+P313	If exposed or concerned: Get medical advice / attention.

This material and its container must be disposed of in a safe way.

The following warnings and precautions apply to this component:

- Pre-Treatment Reagent 2

WARNING:	Contains monothioglycerol.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
Prevention	
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.
P302+P352	IF ON SKIN: Wash with plenty of water.
P332+P313	If skin irritation occurs: Get medical advice / attention.
P362	Take off contaminated clothing and wash before reuse.

This material and its container must be disposed of in a safe way.

The following warnings and precautions apply to this component:

- Pre-Treatment Reagent 1

DANGER:	Contains sodium hydroxide.
H314	Causes severe skin burns and eye damage.
H290	May be corrosive to metals.
Prevention	
P234	Keep only in original container.
P260	Do not breathe mist / vapours / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P301+P330 +P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361 +P353	IF ON SKIN (or hair): Remove / Take off immediately all contaminated clothing. Rinse skin with water / shower.
P363	Wash contaminated clothing before reuse.
P310	Immediately call a POISON CENTER or doctor / physician.
P390	Absorb spillage to prevent material damage.

This material and its container must be disposed of in a safe way.

The following warnings and precautions apply to this component:

- Conjugate

WARNING:	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapours / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P363	Wash contaminated clothing before reuse.

This material and its container must be disposed of in a safe way.

- The Pre-Treatment Reagent 3 contains sodium azide. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.
- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a reagent kit or between reagent kits.**
- Before loading the ARCHITECT B12 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septa MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septa are not used according to the instructions in this package insert.**
  - Prolonged exposure of B12 Pre-Treatment Reagent 1 to air may compromise performance.
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT B12 Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT B12 Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septa and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT B12 assay file must be installed on the ARCHITECT *i* System from an ARCHITECT *i* System assay CD-ROM before performing the assay. For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT B12 assay is pg/mL. An alternate result unit, pmol/L, may be selected for reporting results by editing assay parameter "Result concentration units", to pmol/L. The conversion factor used by the system is 0.7378.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

The following specimen tube types were verified for use with the ARCHITECT B12 assay:

Glass	Plastic
Serum	Serum
	Serum separator
	Lithium heparin plasma separator
	Sodium heparin
	Dipotassium EDTA

The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT B12 assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - hemolyzed
  - obvious microbial contamination
- Performance has not been established for the use of body fluids other than human serum and plasma.
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at > 10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter or
  - they were frozen and thawed.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.
- Transfer clarified specimen to a sample cup or secondary tube for testing.

Storage

- Specimens may be stored on or off the clot, red blood cells, or separator gel for
  - up to 3 days at room temperature or
  - up to 7 days at 2-8°C.
- If testing will be delayed more than 3 days for specimens stored at room temperature or more than 7 days for specimens stored at 2-8°C, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20°C or colder.
- Avoid more than three freeze/thaw cycles.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped frozen (dry ice) -20°C or colder. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 7K61 ARCHITECT B12 Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT B12 Assay file, may be obtained from:
  - ARCHITECT *i* System e-Assay CD-ROM found on www.abbottdiagnostics.com
  - ARCHITECT *i* System Assay CD-ROM
- 7K61-01 ARCHITECT B12 Calibrators
- 7K61-10 ARCHITECT B12 Controls
- 7D82-50 ARCHITECT *i* Multi-Assay Manual Diluent
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional).
- For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT B12 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - Invert the microparticle bottle 30 times.
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
- Load the ARCHITECT B12 Reagent Kit on the ARCHITECT *i* System. Verify that all necessary assay reagents are present. Ensure that septa are present on all reagent bottles. Refer to ARCHITECT Operations Manual, Section 5, for details on how to load reagents.
- Order calibration, if necessary.
- For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
- For information on ordering patient specimens, calibrators and controls and general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.

- The minimum sample cup volume is calculated by the system and is printed on the Order List Report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test.
  - Priority: 87 µL for the first B12 test plus 37 µL for each additional B12 test from the same sample cup
  - ≤ 3 hours onboard: 150 µL for the first B12 test plus 37 µL for each additional B12 test from the same sample cup
  - > 3 hours onboard: replace with a fresh sample (patient specimens, controls, and calibrators).
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
  - Prepare calibrators and controls.
  - Mix the ARCHITECT B12 Calibrators and Controls by gentle inversion before use.
  - To obtain the recommended volume requirements for the ARCHITECT B12 Calibrators and Controls, hold the bottles **vertically**, and dispense 3 drops of each calibrator or 3 drops of each control into each respective sample cup.
- Load samples
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Specimens with a B12 value exceeding 2000 pg/mL (1476 pmol/L) are flagged with the code ">2000" when working in pg/mL (">1476" when working in pmol/L) and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

- If using the Automated Dilution Protocol, the system performs a 1:3 dilution. The system will use the dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result.
- Manual dilutions should be performed as follows:
  - The suggested dilution for B12 is 1:4.
  - For a 1:4 dilution, add 100 µL of the patient specimen to 300 µL of ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
  - The suggested dilution for specimens that generate repeated (2 or more) "3350 Unable to process test-aspiration error for (Sample Pipettor) at (RV 24)" errors is 1:2.
  - For a 1:2 dilution, add 100 µL of the patient specimen to 100 µL of ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
  - The operator must enter the dilution factor in the patient or control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result. The result before the dilution factor is applied must be greater than 83 pg/mL (61 pmol/L).

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT B12 calibration, test Calibrators A through F in duplicate. Calibrators should be priority loaded.
- Calibration Range: 0-2000 pg/mL (0-1476 pmol/L).
- A single sample of each control level must be tested to evaluate the assay calibration.
  - Order controls as described above.
  - Ensure that assay control values are within the concentration ranges specified in the control package insert.
- Once an ARCHITECT B12 calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT B12 assay is a single sample of all control levels tested once every 24 hours each day of use. Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Control values must be within the ranges specified in the control package insert. If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been adversely affected. Adversely affected test results are invalid, and these samples must be retested. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT B12 assay belongs to method group 1.

RESULTS

The ARCHITECT B12 assay utilizes a 4 Parameter Logistic Curve Fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

Alternate Result Units

- The default result unit for the ARCHITECT B12 assay is pg/mL. When the alternate result unit, pmol/L, is selected, the conversion factor used by the system is 0.7378.
- Conversion Formula:

  (Concentration in pg/mL) x (0.7378) = pmol/L

  (Concentration in pmol/L) / (0.7378) = pg/mL

Flags

- Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- The diagnosis of B12 deficiency cannot be solely based on serum or plasma B12 levels. Further testing for folic acid, intrinsic factor blocking antibodies, holotranscobalamin,[5] homocysteine, and/or methylmalonic acid is suggested for symptomatic patients with hematological or neurological abnormalities.[6,7]
- If the B12 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Hemolysis has been demonstrated to exhibit negative interference in this B12 assay. Hemolyzed specimens should not be analyzed.
- Specimens containing above normal protein concentrations may generate repeated (2 or more) "3350 Unable to process test-aspiration error for (Sample Pipettor) at (RV 24)" errors and should be quantified using the Automated Dilution Protocol or Manual Dilution Procedure (1:2).
- Heterophilic antibodies and rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[17] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- The assay is designed to test human serum and plasma. Specimens tested in other matrices may not give accurate results.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert for specimen limitations.

EXPECTED VALUES

B12 Normals

It is recommended that each laboratory establish its own range, which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) document C28-A2.[18] Serum specimens from 143 individuals with normal mean corpuscular volume, homocysteine, and folate results were assayed for B12 using the ARCHITECT B12 assay. The B12 concentration range for this population was 141 to > 1218 pg/mL (104 to > 899 pmol/L) with a mean of 407 pg/mL (300 pmol/L). The central 95% of the sample population is defined below:

Expected Range	187-883 pg/mL	(138-652 pmol/L)

B12 Indeterminates

Levels above 300 or 400 pg/mL (221 or 295 pmol/L) are rarely associated with B12 deficiency induced hematological or neurological disease, respectively. Further testing is suggested for symptomatic patients with B12 levels between 100 and 300 pg/mL (74 and 221 pmol/L) (hematological abnormalities), and between 100 and 400 pg/mL (74 and 295 pmol/L) (neurological abnormalities).[6,7]

SPECIFIC PERFORMANCE CHARACTERISTICS

Assay results obtained in individual laboratories may vary from the data presented in the following studies.

Precision

The ARCHITECT B12 assay is designed to have a Total CV of ≤ 11% for concentrations in the range of the low, medium, and high controls. A 20-day precision study was performed for the ARCHITECT B12 assay based on guidance from the CLSI document EP5-A2.[19] Testing was conducted at Abbott Laboratories using three ARCHITECT B12 assay reagent lots, two calibrator lots, one control lot, and two instruments. Four levels of controls and panels were assayed in replicates of three at two separate times of day for 20 different days. The data are summarized in the following table.

Instrument	Sample	n	Mean (pg/mL)	Within-Run SD	Within-Run %CV	Within Laboratory Precision (Total) SD	Within Laboratory Precision (Total) %CV
1	Serum Panel	360	262	12.6	4.8	16.3	6.2
	Low Control	354	246	13.8	5.6	16.7	6.8
	Medium Control	355	424	14.3	3.4	16.8	4.0
	High Control	359	890	36.0	4.0	38.9	4.4
2	Serum Panel	357	248	11.6	4.7	13.3	5.4
	Low Control	356	241	10.4	4.3	12.9	5.4
	Medium Control	352	408	13.3	3.3	15.5	3.8
	High Control	355	885	23.9	2.7	29.7	3.4

Accuracy by WHO

A study was conducted to evaluate the accuracy of the ARCHITECT B12 assay using the B12 World Health Organization International Standard 03/178. The assay demonstrated a -3.6% difference from the target value of 480 pg/mL (354 pmol/L).

Sensitivity

Sensitivity is defined as the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) for the ARCHITECT B12 assay. The assay is designed to have an LoQ of ≤ 150 pg/mL (≤ 111 pmol/L). A study conducted based on guidance from CLSI document EP17-A[20] produced an LoB of 83 pg/mL (61 pmol/L), an LoD of 125 pg/mL (92 pmol/L) and LoQ of 125 pg/mL (92 pmol/L).

Specificity

The ARCHITECT B12 assay is designed to have an interference (difference) less than the LoD of the assay with cobinamide, a B12 analogue. The specificity of the ARCHITECT B12 assay was determined by studying the cross reactivity with cobinamide. A human serum specimen at approximately 230 pg/mL (168 pmol/L) was supplemented with cobinamide at 9000 pg/mL and the resulting interference was 4 pg/mL (3 pmol/L).

Interference

At the concentrations listed below, bilirubin (conjugated and unconjugated), total protein, and triglycerides showed less than 10% interference in the ARCHITECT B12 assay for low samples (concentration range: 150 pg/mL to 250 pg/mL (111 pmol/L to 184 pmol/L)) and higher samples (concentration range: > 500 pg/mL (> 369 pmol/L)):

- Bilirubin < 25.1 mg/dL
- Total Protein < 12 g/dL
- Triglycerides < 3325 mg/dL

Hemolyzed specimens should not be analyzed; refer to the **LIMITATIONS OF THE PROCEDURE** section of this package insert.

Accuracy by Correlation

A study was conducted based on guidance from CLSI document EP9-A2.[21] Three hundred and twenty nine serum specimens were tested for the determination of B12 using the ARCHITECT B12 assay and a commercially available diagnostic kit. The specimen testings are shown in the following table*.

Abbott ARCHITECT B12 vs AxSYM B12

Method	Number of Specimens	Intercept	Slope	Correlation Coefficient
Least Squares Linear Regression	329	-2.05	1.01	0.99
Passing-Bablok Linear Regression[22]	329	21.96	0.95	0.99

In this evaluation, serum specimens tested ranged from 113 to 2769 pg/mL (83 to 2043 pmol/L) by the ARCHITECT B12 assay, and from 93.5 to 2655.8 pg/mL (69.1 to 1959.5 pmol/L) by the comparator assay.

* Representative data, results in individual labs may vary from these data.

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February 2013

APPENDIX V

- **NHS Ethics for ENRICC study**

Telephone: 0131 536 9022
Facsimile: 0131 536 9346

01 June 2007

Dr David C. Wilson
Senior Lecturer and Honorary Consultant in Paediatrics
University of Edinburgh
Child Life and Health University of Edinburgh,
20 Sylvan Place
Edinburgh
EH9 1UW

Dear Dr Wilson

Full title of study: **Nutritional Risk in Children and Young People with Cancer: Determinants and Screening Tools for both the Short-term and the Long-term**

REC reference number: **06/S1104/52**

Thank you for your letter of 28 May 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered by the Chair on behalf of LREC 4 on 31 May 2007

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	Revised	
Investigator CV		04 January 2007
Protocol	1.3	28 May 2007
Covering Letter		09 January 2007

Questionnaire: Complementary & alternative therapy survey	1	28 April 2007
Letter of invitation to participant	parent revised prospective	13 April 2007
Letter of invitation to participant	Revised Retro	12 April 2007
GP/Consultant Information Sheets		04 January 2007
Participant Information Sheet: & Parent Prospective Study	1.0	03 January 2007
Participant Information Sheet: & Parent Retrospective Study	1.0	03 January 2007
Participant Information Sheet: parent & child prospective study PIS	revised prospective	13 April 2007
Participant Information Sheet: PIS parent & Child retrospective study PIS	revised retrospective	12 April 2007
Participant Consent Form: child 13-16 prosp	1 prosp	26 April 2007
Participant Consent Form: Child 8-12 prosp	1 prosp	26 April 2007
Participant Consent Form: Parent PCF prosp	2 prosp	26 March 2007
Participant Consent Form: child <8 CF prosp	1 prosp	26 April 2007
Participant Consent Form: child 13-16 retros	1 retro	26 April 2007
Participant Consent Form: child 8-12 CF retros	1 retro	26 April 2007
Participant Consent Form: Child < 8 CF retrosp	1 retro	26 April 2007
Participant Consent Form: Parent CF retro	2	26 March 2007
Response to Request for Further Information		28 May 2007
Summary Doc	Responses	28 May 2007
PedsQL Teen Report (ages 13-18)	3.0	
Overall Health Questionnaire		
Food Diaries	1	03 January 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Feedback on the application process

Now that you have completed the application process you are invited to give your view of the service you received from the National Research Ethics Service. If you wish to make your views known please use the feedback form available on the NRES website at:

https://www.nresform.org.uk/AppForm/Modules/Feedback/EthicalReview.aspx

We value your views and comments and will use them to inform the operational process and further improve our service.

06/S1104/52	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Mike Logan
Chair

Email: joyce.clearie@lhb.scot.nhs.uk

Enclosures: *Standard approval conditions [SL-AC1 for CTIMPs, SL-AC2 for other studies]*

Copy to: [R&D office for NHS care organisation at lead site]

Lothian Local Research Ethics Committee 04

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	06/S1104/52	**Issue number:**	0	**Date of issue:**	01 June 2007
Chief Investigator:	Dr David C. Wilson				
Full title of study:	Nutritional Risk in Children and Young People with Cancer: Determinants and Screening Tools for both the Short-term and the Long-term				

This study was given a favourable ethical opinion by Lothian Local Research Ethics Committee 04 on 31 May 2007. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes* (1)

Approved by the Chair on behalf of the REC:

..... *(Signature of Chair/Co-ordinator)*
(delete as applicable)

..... (Name)

(1) *The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.*

ACCORD

Academic and Clinical Central Office for Research and Development

NHS Lothian

The Queen's Medical Research Institute
47 Little France Crescent, Edinburgh EH16 4TJ
Research and Development Management Suite

15 June 2007

Dr David Wilson
University of Edinburgh
Child Life and Health
20 Sylvan Place
Edinburgh
EH9 1UW

Dear Dr Wilson

Study Title: **Nutritional Risk in Children and Young People with Cancer: Determinants and Screening Tools for both the Short-term and the Long-term**
REC: **06/S1104/52**

Under the requirements of the Scottish Executive Health Department's Research Governance Framework for Health and Community Care, the University of Edinburgh and NHS Lothian Health Board agree in principle to act as co-sponsors for this project. Co-sponsorship is subject to you obtaining a favourable ethical opinion and NHS Lothian R&D management approval.

As Chief Investigator, you must ensure that the study does not commence until all applicable approvals have been obtained.

Please note this letter should not be considered as NHS Lothian R&D management approval.

Following receipt of all relevant approvals, you should ensure that any amendments to the project are notified to the co-sponsors, REC, MHRA (where appropriate) and NHS Lothian R&D Office.

Yours sincerely

Tina McLelland
Research Governance Manager
Research & Development Office
NHS Lothian

Marise Bucukoglu
Associate Director
Edinburgh Clinical Trials Unit
University of Edinburgh

cc NHS R&D Office
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Associate Director (Governance): **Marise Bucukoglu**

edinburgh bioQuarter

APPENDIX VI

- **PTH protocol**

- **Vitamin D protocol**

IMMULITE® 2000 Intact PTH

English

Intended Use: For *in vitro* diagnostic use with the IMMULITE 2000 Systems Analyzers — for the quantitative measurement of intact parathyroid hormone (parathyrin, PTH) in EDTA plasma and serum, as an aid in the differential diagnosis of hypercalcemia and hypocalcemia.

Catalog Number: **L2KPP2** (200 tests), **L2KPP6** (600 tests)

Test Code: **iPT** Color: **Dark Green**

Summary and Explanation

Parathyroid hormone (parathyrin, PTH), a single-chain polypeptide (with a molecular mass of approximately 9,500 daltons) containing 84 amino acids, exerts significant influence in the maintenance of optimal calcium ion concentrations. PTH raises serum ionized calcium levels through direct action on bone and the kidneys: it increases the rate of calcium ion flow from bone to the extracellular fluid, and increases both the renal tubular reabsorption of ionized calcium and the renal excretion of phosphate. Long-term regulation of total body calcium by PTH occurs through its stimulation of vitamin D metabolism, which results in enhanced intestinal absorption of ionized calcium.[1]

In healthy individuals, PTH is secreted in response to circulating calcium ion levels. Any dip below an individual's normal level triggers a pronounced increase in PTH secretion. Calcium levels returning to normal exert a negative feedback effect, thus inhibiting PTH secretion by the parathyroid glands.[1]

PTH undergoes proteolysis to a lesser extent in the parathyroid glands but mostly peripherally – especially in the liver but also in the kidneys and bone – to yield N-terminal fragments and longer lived C-terminal and midregion fragments. The N-terminal fragment contains the region that confers bioactivity. C-terminal and N-terminal fragments are initially generated in equivalent amounts, but the N-terminal fragments disappear rapidly.

The C-terminal fragment has a half-life of several hours. In renal failure, C-terminal fragment clearance by glomerular filtration is impaired, so that high levels are found. C-terminal assays (as well as midregion assays) are consequently likely to be especially unreliable in chronic renal failure, where increased PTH is typically just a reflection of impaired renal clearance.[1,2]

For the intact hormone, the *in vivo* half-life is 2 to 5 minutes.[3] Intact PTH clearance is accomplished by both peritubular uptake and glomerular filtration followed by reabsorption. In normal renal function, intact PTH is the greatest part of circulating PTH-like bioactivity[4] and is present in the circulation at concentrations of 10^{-11} to 10^{-12} mol/L.[2]

In hypercalcemia due to primary hyperparathyroidism or to ectopic PTH production (pseudohyperparathyroidism), the majority of patients have elevated PTH levels. By contrast, in hypercalcemia due to malignancy or other causes, the concentration of PTH in circulation is typically low or within normal reference range limits. PTH levels are also characteristically high in secondary hyperparathyroidism – usually associated with renal failure – as a result of constant stimulation of the parathyroid gland by low calcium levels. Hypocalcemia accompanied by a low PTH level, on the other hand, is to be expected in hypoparathyroidism, either postsurgical or idiopathic.[2,5,6]

Immunoassays specific for various PTH fragments have been developed. Most rely on antisera specific for a discrete region: the C-terminal, N-terminal, or midmolecule. The antisera employed in such assays recognize not only the specific region, but similar fragments as well.[1,4]

Recent assays for intact PTH have the necessary sensitivity for detecting circulating intact PTH in normals and for discriminating between normals and those with primary hyperparathyroidism. These assays also appear to discriminate better between primary hyperparathyroidism and hypercalcemia of malignancy compared

with previous assays, and do so virtually without any significant overlap between these groups.[4]

Much improved clinical sensitivity is reported for PTH assays when dynamic reference intervals (based on a range of serum PTH values obtained by acute modification of serum calcium concentrations in healthy subjects) are used, rather than a gaussian reference range (based on PTH values seen in normocalcemic individuals). Using an immunoradiometric assay for intact PTH and applying a dynamic reference range, Lepage, *et al.* obtained average clinical sensitivity of up to 100 percent with primary hyper- and hypoparathyroid samples. Moreover, only the intact PTH assay allowed complete separation between primary hyperparathyroid and nonparathyroidal hypercalcemic patients.[7]

Principle of the Procedure

IMMULITE 2000 Intact PTH is a solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay.

Incubation Cycles: 1 × 60 minutes.

Specimen Collection

Because of the nocturnal rise in intact PTH levels, samples should be collected in the morning, after 7 a.m., preferably after an overnight fast. (A study by Logue *et al.* suggests that sampling after 10 a.m. may optimize discrimination between normal subjects and patients with mild primary hyperparathyroidism.[8])

Collect blood by venipuncture into plastic EDTA plasma tubes or plain plastic serum tubes (with or without gel barrier), avoiding hemolysis. Both plasma and serum should be separated from the cells as soon as possible.

For EDTA plasma, keep specimens cold (2–8°C) throughout the collection and separation process. Separate the plasma from the cells, using a refrigerated centrifuge, if possible. Note that EDTA collection tubes must be filled to their capacity. Failure to completely fill the tube will result in excess concentration of EDTA which will interfere with the assay, causing a false depression of values.

For serum, allow the specimens to clot at room temperature. Separate the serum from the cells, using a refrigerated centrifuge, if possible.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Lipemic, hemolyzed, icteric or grossly contaminated samples may give erroneous results.

The use of an ultracentrifuge is recommended to clear lipemic samples.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants. IMMULITE 2000 Intact PTH has been tested with plastic BD Vacutainer™ tubes (plain serum, SST and EDTA). It has not been tested with all possible variations of tube types.

Volume Required: 50 µL serum or plasma.

Storage: Samples can be stored at 2–8°C for up to 8 hours after collection. For longer storage, aliquot and freeze up to 2 months at –20°C.

Warnings and Precautions

For *in vitro* diagnostic use.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. Labels on the inside box are needed for the assay.

Intact PTH Bead Pack (L2PP12)
With barcode. 200 beads, coated with affinity-purified murine monoclonal anti-PTH (44-84) antibody. Stable at 2–8°C until expiration date.
L2KPP2: 1 pack. **L2KPP6:** 3 packs.

Intact PTH Reagent Wedge (L2PPA2)
With barcode. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to affinity-purified goat polyclonal anti-PTH (1-34) in buffer, with preservative. Stable at 2–8°C until expiration date.
L2KPP2: 1 wedge. **L2KPP6:** 3 wedges.

Before use, tear off the top of the label at the perforations, without damaging the barcode. Remove the foil seal from the top of wedge; snap the sliding cover down into the ramps on the reagent lid.

Intact PTH Adjustors (LPHL, LPHH)
Two vials (Low and High) of lyophilized, synthetic human intact PTH in a buffered matrix. Reconstitute each vial with **2.0 mL** distilled or deionized water, then place Adjustors on ice immediately. Mix by gentle, intermittent swirling. Use *only freshly reconstituted* Adjustors for each assay. *Do not freeze.*
L2KPP2: 3 sets. **L2KPP6:** 5 sets.

Before making an adjustment, place the appropriate Aliquot Labels (supplied with the kit) on test tubes so that the barcodes can be read by the on-board reader.

Kit Components Supplied Separately

Intact PTH Sample Diluent (L2PHZ)
For on-board dilution of high samples. 25 mL of concentrated (ready-to-use) PTH-free buffer matrix. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.

Barcode labels are provided for use with the diluent. Before use, place an appropriate label on a 16 × 100 mm test tube, so that the barcodes can be read by the on-board reader
L2PHZ: 3 labels

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
LRXT: Reaction Tubes (disposable)
L2ZT: 250 Sample Diluent Test Tubes (16 × 100 mm)
L2ZC: 250 Sample Diluent Tube Caps

LPHCM: Bi-level Intact PTH control, in a buffered matrix.

Also Required
Distilled or deionized water; test tubes; controls.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE 2000 Systems Operator's Manual.

See the IMMULITE 2000 Systems Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

Recommended Adjustment Interval: 4 weeks.

Quality Control Samples: Use controls or patient sample pools with at least two levels (low and high) of intact PTH.

Expected Values

A reference range study was conducted on matched EDTA plasma and serum samples from 88 apparently healthy volunteers, collected into BD plastic Vacutainer™ tubes. The matched samples were analyzed on IMMULITE/IMMULITE 1000 Intact PTH and on IMMULITE 2000 Intact PTH assays. Analysis of the data indicated no statistically significant difference between platforms, although there was a clear difference in reference ranges between EDTA plasma and serum. The reference ranges suggested by this study for IMMULITE/IMMULITE 1000 Intact PTH and IMMULITE 2000 Intact PTH for both EDTA plasma and serum are shown in the following table.

	Median	Central 95% Range (pg/mL)
EDTA Plasma	38	16 – 87
Serum	30	11 – 67

The reference range for serum is in good agreement with a previous multi-site reference range study, conducted with IMMULITE 2000 Intact PTH on 255 serum samples from apparently healthy subjects. This population yielded a median of 32 pg/mL. The reference range suggested by this study is:

12 – 65 pg/mL (1.3 – 6.8 pmol/L).

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

Limitations

The assay is intended strictly as an aid in the differential diagnosis of hypercalcemia and hypocalcemia, *not* for the diagnosis or management of malignancy.

Because of the physiological relationship between circulating calcium and PTH, it is always important to interpret PTH results in the light of total or ionized calcium levels.[9,10] The finding of a persistently high-normal calcium accompanied by a high-normal PTH (alternatively, a low-normal calcium accompanied by a low-normal PTH) warrants further investigation; for the PTH, though itself within normal limits, may still be inappropriately high (or inappropriately low) relative to the circulating calcium level.[11]

Indices of renal function, e.g. creatinine levels; measurement of albumin, as an adjunct to measurement of *total* calcium levels;[12-15] and determinations of phosphorus,[14,16] chloride,[12] nephrogenous cyclic AMP[14,17,18] and possibly calcitonin,[14] may also (in certain circumstances) aid in the interpretation of PTH and calcium results. It should also be remembered that hypercalcemia and hypocalcemia may be secondary to disordered vitamin D metabolism.[19]

Lipemic, hemolyzed, icteric or grossly contaminated samples may give erroneous results.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1998:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Performance Data

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in pg/mL.

Unless otherwise specified, all performance data were generated with EDTA plasma samples.

Conversion Factor:
pg/mL × 0.1053 → pmol/L

Calibration Range: Up to 2,500 pg/mL (263 pmol/L).

The assay is traceable to an internal standard manufactured using qualified materials and measurement procedures.

Analytical Sensitivity: 3.0 pg/mL (0.3 pmol/L).

High-Dose Hook Effect: None up to 500,000 pg/mL (50,000 pmol/L).

Intraassay Precision: Statistics were calculated for samples from the results of 20 replicates in a single run. (See "Intraassay Precision" table.)

Interassay Precision: Statistics were calculated for samples assayed in 10 different runs. (See "Interassay Precision" table.)

Linearity: Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Samples spiked 1 to 19 with three intact PTH solutions (900, 3,600 and 7,200 pg/mL) were assayed. (See "Recovery" table for representative data.)

Specificity: The antibody is highly specific for intact PTH. (See "Specificity" table.)

Antibodies used in the kit were purified by affinity chromatography to achieve specificity for well-defined regions of the intact PTH molecule. The antibodies immobilized to the solid phase (coated bead) are specific for the C-terminal region (44-84) and have no detectable crossreactivity to the N-terminal region (1-34). Conversely, the enzyme-labeled antibody recognizes only the N-terminal region, and has no detectable crossreactivity to C-terminal or midmolecule regions. Accordingly, the assay, which requires binding by both enzyme-labeled and solid-phase antibodies, is able to recognize only intact PTH and very large PTH fragments that are nearly as long as intact PTH itself. One such fragment, PTH 7–84, exhibits significant crossreactivity (44.8%)[20] in the IMMULITE 2000 PTH assay.

Bilirubin: Presence of conjugated and unconjugated bilirubin in concentrations up to 200 mg/L may cause a depression of values. (See "Bilirubin" table.)

Hemolysis: Presence of hemoglobin in concentrations up to 513 mg/dL may cause a depression of values. (See "Hemolysis" table.)

Lipemia: Presence of triglycerides in concentrations up to 3,000 mg/dL may cause a depression of values. (See "Lipemia" table.)

Method Comparison 1: The assay was compared to a chemiluminescent immunometric assay (Kit A) on 79 serum samples. (Concentration range: approximately 3 to 1,300 pg/mL. See graph 1.) By linear regression:

(IML 2000) = 0.90 (Kit A) – 6.9 pg/mL
r = 0.904

Means:
224 pg/mL (IMMULITE 2000)
255 pg/mL (Kit A)

Method Comparison 2: The assay was also compared to a radioimmunometric assay (Kit B) on 99 serum samples. (Concentration range: approximately 3 to 1,300 pg/mL. See graph 2.) By linear regression:

(IML 2000) = 0.98 (Kit B) + 5.5 pg/mL
r = 0.889

Means:
237 pg/mL (IMMULITE 2000)
237 pg/mL (Kit B)

References

1) Armitage EK. Parathyrin (parathyroid hormone): metabolism and methods for assay. Clin Chem 1986;32:418-24. 2) Blind E, Schmidt-Gayk H, Scharla S, Flentje D, Fischer S, Goehring U, Hitzler W. Two-site assay of intact parathyroid hormone in the investigation of primary hyperparathyroidism and other disorders of calcium metabolism compared with a midregion assay. J Clin Endocrinol Metab 1988;67:353-60. 3) Kao PC, Grant CS, Klee GG, Khosla S. Clinical performance of parathyroid hormone immunometric assays. Mayo Clin Proc 1992;67:637-45. 4) Measuring the PTH level. Lancet 1988 (Jan 16);94-5. 5) Christensen MS. Radioimmunoassay of human parathyroid hormone. Dan Med Bull 1979;26:157-73. 6) Hawker CD, Di Bella FP. Radioimmunoassays for intact and carboxyl-terminal parathyroid hormone: clinical interpretation and diagnostic significance. Ann Clin Lab Sci 1980;10:76-87. 7) Lepage R, Whittom S, Bertrand S, Bahsali G, D'Amour P. Superiority of dynamic over static reference intervals for intact, midmolecule, and C-terminal parathyrin in evaluating calcemic disorders. Clin Chem 1992;38:2129-35. 8) Logue FC, Fraser WD, O'Reilly DStJ, Beastall GH. The circadian rhythm of intact parathyroid hormone (1-84) and nephrogenous cyclic adenosine monophosphate in normal men. J Endocrinol 1989;121:R1-R3. 9) Bowers GN, Brassard C, Sena SF. Measurement of ionized calcium in serum with ion-selective electrodes. Clin Chem 1986;32:1437-47. 10) Halvorsen JF, Gautvik VT, Gautvik KM. Improved diagnosis of primary hyperparathyroidism by defining the inverse relationship between serum immunoreactive parathyroid hormone and calcium. Scand J Clin Lab Invest 1986;46:435-42. 11) Hawker CD, Clark SW, Martin KJ, Slatopolsky E, Di Bella FP. Radioimmunoassay of parathyroid hormone: clinical utility and interpretation. In: Thompson NW, Vinik AI, editors. Endocrine surgery update. New York: Grune & Stratton, 1983: 321-40. 12) Boyd JC, Ladenson JH. Value of laboratory tests in the differential diagnosis of hypercalcemia. Am J Med 1984;77:863-72. 13) Groth TL, Ljunghall S, De Verdier C-H. Optimal screening for patients with hyperparathyroidism with use of serum calcium observation; a decision-theoretical analysis. Scand J Clin Lab Invest 1983;43:699-707. 14) Kleeman K, Kleeman CR. Parathyroid hormone and calcitonin. Contemp Endocrinol 1985;2:247-344. 15) Orwoll ES, Meier D. Alterations in calcium, vitamin D, and parathyroid hormone physiology in normal men with aging: relationship to the development of senile osteopenia. J Clin Endocrinol Metab 1986;63:1262-9. 16) Kao PC. Parathyroid hormone assay. Mayo Clin Proc 1982;57:596-7. 17) Heath H. Tests of parathyroid function: utility and limitations. American Association for Clinical Chemistry: Continuing Education in Endocrinology and Metabolism. Endo (Feb)

Vitamin D protocol

CLINICAL BIOCHEMISTRY

Department of Laboratory Medicine, LUHD

27th August 2012

Dear Colleague

VITAMIN D REQUESTS – IMPORTANT ANNOUNCEMENT

You will recall that early last year Glasgow Royal Infirmary placed restrictions on the use of their Vitamin D assay service in order to cope with an ever increasing workload. These pressures remain and, in order to manage a continuing increase in demand, the labour-intensive Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) assay is to be replaced by a fully automated Vitamin D immunoassay method for routine measurements.

While 25OH Vitamin D3 levels obtained by immunoassay correlate well with those measured by LC-MS/MS, the immunoassay method has reduced cross reactivity with 25OH Vitamin D2 compared with 25OH Vitamin D3. Therefore when the assessment of Vitamin D status in patients receiving Vitamin D2 supplements is required, it will be necessary to request measurement by the LC-MS/MS assay which measures 25OH Vitamin D2 and 25OH Vitamin D3 separately.

We anticipate that the LC-MS/MS assay should only be necessary for a small number of patients. When required, a written request form must always be submitted to the laboratory and the requirement for 25OH Vitamin D2 assay clearly indicated. It will not be possible to make this request on TRAK or on the future GP electronic ordering system, both of which will be configured to request measurement by immunoassay only.

Please note that Vitamin D assays are not indicated for patients being treated with alphacalcidol or calcitriol and that the restrictions on the frequency of testing already in place continue to apply (normally once a year in adults).

The new assay will be introduced on Monday 3rd September 2012. If you require further information about this change please contact the Duty Biochemist in your local laboratory.

Yours sincerely

JP Ashby
Specialty Lead for Clinical Biochemistry

APPENDIX VII

- **Calcium protocol**
- **Magnesium protocol**
- **Phosphate protocol**

Calcium protocol

PRE-EXAMINATION

Reception Procedures – **LP-REC-E-RecProc**

RHSC user Handbook **PD-GEN-A-USERHND**

SAMPLE REQUIREMENTS:

Serum or heparinised plasma may be analysed. EDTA / fluoride oxalate tubes are unsuitable.
A random 'spot' urine specimen is required for urine analysis.
Minimum volume is 2.6uL [+25uL dead volume].
Store all samples at 4^{o}C until analysis.

EXAMINATION

PRINCIPLE:

Arsenazo-III dye reacts with calcium in an acid solution to form a blue-purple complex. The colour developed is measured at 660 nm and is proportional to the calcium concentration in the sample.

EQUIPMENT AND CONSUMABLES:

Abbott Architect c8000 **ON BOARD MANUAL**
Calcium procedure: uploaded from assay disc. **LI-BIO-E-CAKI#Arch**

REAGENTS:

Store manufacturer's kits at 15-30°C. **LI-BIO-E-CaKI#Arch**

(1) REAGENT 1 Abbott Cat no 3L79-21
Use as supplied. Stable on c8000 analyser for 30 days. Remove air bubbles.

(2) REAGENT 2 Abbott Cat no 3L79-21
Use as supplied. Stable on c8000 analyser for 30 days. Remove air bubbles.

CALIBRATION:

The method must be calibrated every 30 days and when a new batch of reagent is used. For full details see the Abbott Architect systems operation manual and the protocol for calibrating the Abbott Architect ci16200.

- MCC Multiconstituent Calibrator, Abbott Cat No 1E65 **LI-BIO-E-MCCKI**
  Use as supplied. Stable for 2 weeks. Upload values from calibration memory stick.

QUALITY CONTROL:

Run quality control samples for plasma calcium every 4 hours and after calibration.

Run quality control samples for urine calcium as required and after calibration. For full details see the Abbott Architect systems operation manual**.**

- Bio-Rad Multiqual level 1 (Prod. No. 697) **LI-BIO-E-MultiqualKI**
- Bio-Rad Multiqual level 2 (Prod. No. 698) **LI-BIO-E-MultiqualKI**
- Level 3 Bio-Rad Multiqual level 3 (Prod. No. 699) **LI-BIO-E-MultiqualKI**

  Enter values from kit insert. Use as supplied. Stable for 2 weeks in fridge.

- Liquichek Urine level 1 (Prod. No. 397) **LI-BIO-E-LiqUrineKI**
- Liquichek Urine level 2 (Prod. No. 398) **LI-BIO-E-LiqUrineKI**

  Enter values from kit insert. Use as supplied. Stable for 30 days in fridge.

PROCEDURE:

For details see the protocol for running patient samples on the Abbott Architect c8000 and the Architect onboard operation manual. **LI-BIO-E-ArchSamp**

If sample appears haemolysed, icteric or lipaemic, request serum indices.
LP-BIO-E-INDICES#Arch

CALCULATION:

Direct down-load of results to computer.

EXPECTED PERFORMANCE:

(1) Assay is linear up to 6 mmol/L. [6.00 mmol/L for urine].

(2) Plasma results >6 mmol/L may be manually diluted using saline. The dilution factor should be entered in the patient order screen to automatically correct the result otherwise the result must be manually multiplied.
There is no Automated Dilution Protocol for plasma.
Urine results >6.00 mmol/L may be diluted using Automated Dilution Protocol set at 1:2 [NB standard dilution for urines is 1:1] **LI-BIO-E-CAKI#Arch**

(3) Limit of detection 0.125mmol/L; Limit of Quantitation 0.25mmol/L for plasma and urine.

(4) Indices interference– please refer to 'Analytes affected by Haemolysis, Lipaemia, Icterus and Delay' **LI-BIO-E-HAEM#Arch**

UNCERTAINTY OF MEASUREMENT:

The assay has a CV of 3% on the analyser.
Calcium in the serum is largely protein bound. Therefore venous stasis during sample collection can lead to increased calcium levels.
Results are not significantly affected by haemolysis, icteria or lipaemia.

Interference from medications and endogenous substances may affect results (Ref2).

POST-EXAMINATION

QUALITY CONTROL VERIFICATION:

Check the Calcium [plasma and urine] QC charts on the Architect c8000.
Action limits defined on the Architect QC charts are set at ± 2SD. **LI-BIO-E-SDLIMIT**
Refer to Acceptance Section of IQC Instruction sheet. **QI-GEN-E-IQC**
If the QC results are outside the action limits given on the chart, record action taken on analyser IQC log or IQC failure form **QF-GEN-E-IQCfail**

RESULT REPORTING AND AUTHORISATION

Results are automatically downloaded into the computer via AMS. **LP-GEN-E-ILABS** **LP-GEN-E-AMS**

For results above the interference limits, do not report results but add comment as appropriate eg HM, LP or IC. **LI-BIO-E-HAEM#Arch**

Technical authorisation to level S **LP-GEN-E-CHECK**

Telephone any urgent result(s) and any plasma result <1.90 mmol/L or >3.00 mmol/L.

LP-GEN-E-Phone **LI-BIO-E-PHONE#Limits**

Urine: calcium:creatinine ratio automatically calculated.

All results are clinically validated by Duty Biochemist.

CLINICAL INTERPRETATION:

Laboratory Handbook **[PD-GEN-A-USERHND]**
Paediatric reference range in-house data (updated 3/7/07) and Abbott comparisons. **LI-GEN-E-LIBRARY**

Plasma

	Reference range (mmol/L)
Neonates <7 days	1.6 – 2.7
Neonates >7 days	2.3 – 3.0
Infants	2.3 - 2.8
Children	2.2 - 2.7

Plasma calcium reference ranges from in-house data.

Hypocalcaemia may be due to low dietary calcium, inadequate calcium absorption (eg vitamin D deficiency) or hypoparathyroidism. Total (but not ionised) plasma calcium may also be low when plasma albumin is low.
Hypercalcaemia is much rarer in children and may be due to hyperparathyroidism, malignant disease, vitamin D toxicity or hypocalciuric hypercalcaemia (an inherited defect in the calcium-sensing receptor).

Urine

	Reference range (mmol/mmol creatinine)
Infants <6 months	<2.4
Infants >6 months	<2.2
Children, 1 – 2 years	<1.5
Children >2 years	<0.6
	[Equivocal range 0.6 – 0.8]

Urine calcium reference ranges for Infants < 6months from ref (4). Ranges for infants >6 months and children 1 – 2 years from ref (5). Range for children >2 years from ref (6), validated by in-house data collected September 2000. In children, the calcium:creatinine ratio in second-morning 'spot' urine samples accurately reflects 24h urine calcium excretion.

Urinary calcium:creatinine ratio is part of the paediatric urinary stone screen. Its measurement may be of value in nephrocalcinosis, stone formation and in diagnosing hypocalciuric hypercalcaemia.

EQAS:

Participation in UKNEQAS General Chemistry and General Urine Chemistry scheme. **LP-GEN-E-EQAS**

sCIENTIFIC REFERENCES:

(1) Kessler G, Wolfman M. Automated procedure for the simultaneous determination of calcium and phosphorus. Clin Chem 1964 ; 10 : 686-703.

(2) Gosling P. Analytical reviews in clinical biochemistry : calcium measurement. Ann Clin Biochem 1986 ; 23 : 145-156.

(3) Corns CM, Ludman CJ. Some observations on the nature of the calcium-cresolphthalein complexone reaction and its relevance to the clinical laboratory. Ann Clin Biochem 1987 ; 24 : 345-351.

(4) Sargent JD, Stukel TA, Kresel J, Klein RZ. Normal values for random urinary calcium to creatinine ratios in infancy. J Pediatr 1993 ; 123 : 393-397.

(5) Matos V, van Melle G, Boulat O, Markert M, Bauchmann C, Guignard J-P. Urinary phosphate/creatinine, calcium/creatinine and magnesium/creatinine ratios in a healthy pediatric population. J Pediatr 1997;131:252-257.

(6) Shaw NJ, Wheeldon J, Brocklebank JT. Indices of intact serum parathyroid hormone and renal excretion of calcium, phosphate, and magnesium. Arch Dis Child 1990;65:1208-1211.

(7) Effect of Drugs on Clinical Laboratory Tests. Ed DSYoung. AACC Press 4th edition 1995.

(8) Source Data for RHSC Paediatric Reference Ranges (1999-) Vol 1 General Section

(9) Source Data for RHSC Paediatric Reference Ranges (2011-) Vol 4 Abbott comparison data

Magnesium Protocol

Procedure:

Procedure must be carried out according to manufacturer's instructions.
Follow normal laboratory procedures to contain risk.

Work area:

Main Laboratory at the RHSC.

PRE-EXAMINATION

Reception Procedures **REC-E4**

RHSC user Handbook **PD-GEN-E-USERHND#RHSC**

SAMPLE REQUIREMENTS:

Lithium heparin plasma or serum or random 'spot' urine required. EDTA blood is unsuitable.
Samples delayed >12h in transit are unsuitable. [Unreferenced – historical]

Minimum volume is 2.4uL [+25uL dead volume] for plasma and urine.
Store all samples at 4^{o}C until analysis.

EXAMINATION

PRINCIPLE:

This method utilizes an arsenazo dye which binds preferentially with magnesium. The absorbance of the arsenazo magnesium complex is measured at 572 nm and is proportional to the concentration of magnesium present in the sample. Calcium interference is prevented by incorporation of a calcium-chelating agent.

EQUIPMENT AND CONSUMABLES:

Abbott Architect c8000 **ON BOARD MANUAL**
Magnesium procedure: uploaded from assay disc. **KIT84**

REAGENTS:

Store manufacturer's kits at 2-8°C. **KIT84**

(3) REAGENT 1 Abbott Cat no 3P68-21
Use as supplied. Remove air bubbles. Stable on c8000 analyser for 21 days.

CALIBRATION:

The method must be calibrated every 30 days, and with lot number change. For full details see the Abbott Architect systems operation manual and the protocol for calibrating the Abbott Architect c8000.

MCC Multiconstituent Calibrator, Abbott Cat No 1E65 **KIT85**

Use as supplied. Store at 2-8°C. Stable for 1 week.
Upload values from calibration memory stick.

QUALITY CONTROL:

Run quality control samples every 4 hours and after calibration. Run quality control samples for urine magnesium as required and after calibration. For full details see the Abbott Architect systems operation manual.

- Bio-Rad Multiqual level 1 (Prod. No. 697) **KIT30**
- Bio-Rad Multiqual level 2 (Prod. No. 698) **KIT30**
- Bio-Rad Multiqual level 3 (Prod. No. 699) **KIT30**

Use as supplied. Stable for 1 week in fridge.

- Liquichek Urine level 1 (Prod. No. 397) **KIT105**
- Liquichek Urine level 2 (Prod. No. 398) **KIT105**

Use as supplied. Stable for 30 days in fridge.

PROCEDURE:

For details see the protocol for running patient samples on the Architect onboard operation manual.

If sample appears haemolysed, icteric or lipaemic, request serum indices. **BIO-E-22**

CALCULATION:

Direct download of results to computer.

EXPECTED PERFORMANCE:

(5) Assay is linear up to 3.90mmol/L [10.85mmol/L for urine].

(6) Plasma results >3.90 mmol/L may be manually diluted using saline. The dilution factor should be entered in the patient order screen to automatically correct the result otherwise the result must be manually multiplied.
There is no Automated Dilution Protocol for plasma.
Urine results >10.85 mmol/L may be diluted using Automated Dilution Protocol set at 1 in 10 [NB standard dilution for urines is 1:5] **KIT84**

(7) Limit of Detection is 0.063mmol/L [0.313mmmol/L for urine]; Limit of Quantitation is 0.27mmol/L [0.67mmol/L for urine].

(8) Indices interference– please refer to 'Analytes affected by Haemolysis, Lipaemia, Icterus and Delay' **BIO-E-6**

(9) Urine acidification is NOT required for random 'spot' urine specimens. [Ref 1]

UNCERTAINTY OF MEASUREMENT:

The assay has a CV of 4% on the analyser.
Icterus and lipaemia have no significant affect on magnesium results. Haemolysis can affect results.

Interference from medications and endogenous substances may affect results [Ref 2].

POST-EXAMINATION

QUALITY CONTROL VERIFICATION:

Check the Magnesium [plasma and urine] QC charts on the Architect c8000.
Action limits defined on the Architect QC charts are set at ± 2SD.
The SD limit may be manufacturer or tighter than quoted manufacturer SD limits. **GEN-E21**

Refer to Acceptance Section of IQC Instruction sheet. **GEN-E21**
If the QC results are outside the action limits given on the chart, record action taken on analyser IQC log or IQC failure form **GEN-E20**

RESULT REPORTING AND AUTHORISATION

Results are automatically downloaded into the computer via AMS. **GEN-E11**
LP-GEN-E-AMS

For results above the interference limits, do not report results but add comment as appropriate eg HM, LP or IC. **BIO-E-6**

Technical authorisation to level S **GEN-E7**

Telephone any urgent result(s) and any result >4.0 mmol/L **GEN-E14** **BI**
Samples delayed >12h in transit: Do not report result. Add 'OLD' in iLABS. [No Ref available] **BI**

Urine: magnesium: creatinine ratio automatically calculated. All results are clinically validated by th

CLINICAL INTERPRETATION:

Laboratory Handbook **[PD-GEN-A-USERHND]**
LI-GEN-E-LIBRARY

Reference Ranges

Plasma magnesium (mmol/L) [Refs 3, 4]

All ages	0.5 – 1.0

Urine magnesium *(*mmol/mmol creat) [Refs 4, 5, 6]

Birth – 6m	no range available
6m – 1y	<2.2
1 – 2y	<1.7
2 – 16y	0.2 - 1.1

Magnesium depletion may occur as a result of decreased input (eg dietary deficiency, total parenteral nutrition, intestinal resection) or increased loss, either renal (eg renal tubular acidosis, nephrotoxic drugs) or non-renal (eg chronic diarrhoea or vomiting). Magnesium depletion may cause cardiac arrhythmias and may have widespread effects on other electrolytes. It impairs PTH secretion and may lead to hypocalcaemia especially in babies. [Refs 7, 8]

EQAS:

Participation in UKNEQAS general chemistry and urine schemes. **GEN-E31**

sCIENTIFIC REFERENCES:

(1) In house study. L:\Laboratory Specialities\Combined Lab RHSC\LABFILES\Public\Pat\General Section\Urine acidification study - Ca Phos Mg

(2) Effect of Drugs on Clinical Laboratory Tests. Ed DSYoung. AACC Press 4th edition 1995.

(3) Source Data for RHSC Paediatric Reference Ranges (1999-) Vol 1 General section.

(4) Source Data for RHSC Paediatric Reference Ranges (2011-) Vol 4 Abbott comparison data.

(5) Shaw NJ, Wheeldon J, Brocklebank JT. Indices of intact serum parathyroid hormone and renal excretion of calcium, phosphate, and magnesium. Arch Dis Child 1990; 65:1208-1211.

(6) Matos V, van Melle G, Boulat O, Markert M, Bachmann C, Guignard J-P. Urinary phosphate/creatinine, calcium/creatinine, and magnesium/creatinine ratios in a healthy paediatric population. J Pediatr 1997; 131:252-257.

(7) Ryan MF. The role of magnesium in clinical biochemistry: an overview. Ann Clin Biochem 1991; 28:19-26.

(8) Elin RJ. Assessment of magnesium status. Clin Chem 1987; 33: 1965-70.

Phosphate Protocol

Procedure:

Procedure must be carried out according to manufacturer's instructions.
Follow normal laboratory procedures to contain risk.

Work area:

Main Laboratory at the RHSC.

PRE-EXAMINATION

Reception Procedures **REC-E4**

RHSC user Handbook **PD-GEN-E-USERHND#RHSC**

SAMPLE REQUIREMENTS:

Serum or heparinised plasma is required.
Samples that have been delayed more than 12h in transit are unsuitable.
Urine should preferably be the second morning sample but urine collected at other times of day is acceptable.

For calculation of TmPO4/GFR (= renal threshold for phosphate *or* tubular maximum reabsorption rate of phosphate per litre of glomerular filtrate), paired blood and urine samples are required. Measure phosphate and creatinine in both plasma and urine.

Minimum volume is 2.6uL [+25uL dead volume] for plasma and urine.
Store all samples at 4^{o}C until analysis.

EXAMINATION

PRINCIPLE:

Inorganic phosphate reacts with ammonium molybdate to form a heteropolyacid complex. The use of a surfactant eliminates the need to prepare a protein free filtrate. The absorbance at 340 nm is directly proportional to the inorganic phosphorus level in the sample. Sample blanks must be run to correct for any non-specific absorbance in the sample.
EQUIPMENT AND CONSUMABLES:

Abbott Architect c8000 **ON BOARD MANUAL**

Phosphate procedure: uploaded from assay disc. **KIT92**

REAGENTS:

Store manufacturer's kits at 15-30°C. **KIT92**

(4) REAGENT 1 Abbott Cat no 7D71-22
Use as supplied. Stable on c8000 analyser for 65 days. Remove air bubbles.

(5) REAGENT 2 Abbott Cat no 7D71-22
Use as supplied. Stable on c8000 analyser for 65 days. Remove air bubbles.

CALIBRATION:

The method must be calibrated every 41 days and with lot number change. For full details see the Abbott Architect systems operation manual and the protocol for calibrating the Abbott Architect c8000.
MCC Multiconstituent Calibrator 1 and 2, Abbott Cat No 1E65 **KIT85**
Use as supplied. Stable for 1 week. Upload values from calibration memory stick

QUALITY CONTROL:

Run quality control samples every 4 hours and after calibration. Run quality control samples for urine calcium as required and after calibration. For full details see the Abbott Architect systems operation manual.

- Bio-Rad Multiqual level 1 (Prod. No. 697) **KIT30**
- Bio-Rad Multiqual level 2 (Prod. No. 698) **KIT30**
- Bio-Rad Multiqual level 3 (Prod. No. 699) **KIT30**

Use as supplied. Stable for 1 week in fridge.

- Liquichek Urine level 1 (Prod. No. 397) **KIT105**
- Liquichek Urine level 2 (Prod. No. 398) **KIT105**

Use as supplied. Stable for 30 days in fridge.

PROCEDURE:

For details see the protocol for running patient samples on the Architect onboard operation manual. **LI-BIO-E-ArchSamp**

If sample appears haemolysed, icteric or lipaemic, request serum indices. **BIO-E-22**

CALCULATION:

Direct download of results to computer.

Duty Biochemist: Calculate TmPO4/GFR using spreadsheet (L:\Quality Management\CPA\Lab SOPs Stnds E,F\RHSC Laboratories\Biochemistry General /LF-BIO-E-TmPGFR). **BIO-E-42**

If only urine received, enter assay code BUPHCR and calculate phosphate:creatinine ratio.

EXPECTED PERFORMANCE:

(10) Assay is linear up to 8.17mmol/L [60.14mmol/L for urine].

(11) Plamsa results >8.17 mmol/L may be manually diluted using saline. The dilution factor should be entered in the patient order screen to automatically correct the result otherwise the result must be manually multiplies. There is no Automated Dilution Protocol. **KIT92**

(12) Limit of Detection is 0.08mmol/L, Limit of Quantitation is 0.201mmol/L [1.418mmol/L for urine].

(13) Indices interference– please refer to 'Analytes affected by Haemolysis, Lipaemia, Icterus and Delay' **BIO-E-6**

(14) Samples delayed more than 12h in transit are unsuitable owing to leakage of phosphate from red cells. **BIO-330**

(15) It is not necessary to acidify urine samples for phosphate analysis.

UNCERTAINTY OF MEASUREMENT:

The assay has a CV of 4.6% on the analyser.
Icterus and lipaemia have no significant affect on results. Haemolysis can affect results.

Interference from medications and endogenous substances may affect results (Ref 8).

POST-EXAMINATION

QUALITY CONTROL VERIFICATION:

Check the Phosphate [plasma and urine] QC charts on the Architect c8000.
Action limits defined on the Architect QC charts are set at ± 2SD.
The SD limit may be manufacturer or tighter than quoted manufacturer SD limits. **GEN-E21**

Refer to Acceptance Section of IQC Instruction sheet. **GEN-E21**
If the QC results are outside the action limits given on the chart, record action taken on analyser IQC log or IQC failure form **GEN-E20**

RESULT REPORTING AND AUTHORISATION

Results are automatically downloaded into the computer via AMS. **GEN-E11 LP-GEN-E-AMS**

For results above the interference limits, do not report results but add comment as appropriate eg HM, LP or IC. **BIO-E-6**

Plasma phosphate: Technical authorisation to S
Urine phosphate: Technical authorisation to R **GEN-E7**

Telephone any urgent result(s) **GEN-E14 BIO-E-7**

Samples delayed >12h in transit: Do not report result. Add 'OLD' in iLABS.

Urine: phosphate:creatinine ratio automatically calculated.

All results are clinically validated by Duty Biochemist. **GEN-E7**
TmPO4/GFR calculation: is done by the Duty Biochemist using the calculation spreadsheet. **BIO-E-42**

If only urine has been received (no paired plasma), calculate phosphate:creatinine ratio instead.

CLINICAL INTERPRETATION:

Laboratory Handbook **[PD-GEN-A-USERHND]**
RHSC paediatric reference range source data **[LI-GEN-E-LIBRARY #MAN24]**

Plasma phosphate (mmol/L) reference ranges (from in-house data collected July 1999):

Birth-1mo	See SMMP Clinical Guidelines
1-12 mo	0.8 – 2.6
1-16y	1.0 – 2.1

Urine phosphate:creatinine ratio and TmPO4/GFR reference ranges are from refs 5-7.

Reference ranges for urine phosphate:creatinine (mmol:mmol) ratio:

Birth-1mo	0.4 – 9.3
1 – 6 mo	0.5 – 11.1
6-12 mo	1.2 – 19.0
1 – 2 y	1.2 – 14.0
2 – 3 y	1.2 – 12.0
3 – 5 y	1.2 – 8.0
5 – 7 y	1.2 – 5.0
7 – 10 y	1.2 – 3.6
10 – 14 y	0.8 – 3.2
14 – 17 y	0.8 – 2.7

Reference ranges for TmPO4/GFR (mmol/L):

Birth-1mo	1.48 – 3.43
1 – 6mo	1.48 – 3.30
6mo – 2y	1.15 – 2.60

2 – 15y	1.15 – 2.44
>15y	0.96 – 1.44

Plasma and urine phosphate show a marked circadian rhythm unrelated to meals, with a nadir at about 1100h and a peak at about 0300h with a mean difference of 0.4 – 0.5 mmol/L between nadir and peak. Plasma phosphate measurements are of value in assessing bone and renal function and in monitoring nutritional intake in infants. TmPO4/GFR is of value in the investigation of the aetiology of hypophosphataemia, in certain disorders of phosphate metabolism and in oncology patients treated with chemotherapy (e.g. ifosfamide) known to cause renal phosphate wasting.

EQAS:

Participation in UKNEQAS general chemistry and urine schemes. **GEN-E31**

sCIENTIFIC REFERENCES:

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APPENDIX VIII

- **Oxygen Radical Absorbance Capacity (ORAC) Antioxidant Assay**

- **Thiobarbituric Acid Reactive Substances (TBARS) Assay**

Oxygen Radical Absorbance Capacity (ORAC) Antioxidant Assay

References:

Ou, B et al (2001) Development and validation of an improved oxygen radical absorbance capacity assay using fluroescein as the fluorescent probe. Journal of Agricultural and Food Chemistry, 49 (10), 4619-4626.

Girard-Lalancette, K et al. (2009) Sensitive cell-based assay using DCFH oxidation for the determination of pro- and anti-oxidant properties of compounds and mixtures: Analysis of fruit and vegetable juices. Food Chemistry, 115, 720-726.

The ORAC assay is based on the oxidation of a fluorescent probe (fluorescein) by peroxyl radicals via a classic hydrogen atom transfer (HAT) mechanism. Free radicals are generated by the water souable compound AAPH (2,2'-azobis-2-methyl-propanimidamide). The peroxyl radicals thus generated quench the fluorescence of fluorescein over time. The antioxidants block the peroxyl radical mediated oxidation of fluorescein until all of the antioxidant activity in the sample is exhausted, after which AAPH-generated peroxyl radicals react with and quench the fluorescence of fluorescein. The area under the fluorescence decay curve (AUC) is used to quantify the total peroxyl radical antioxidant activity in a sample and is compared to a standard curve obtained using various concentrations of the water soluble vitamin E analogue Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Unlike other antioxidant assays, the fluorescent ORAC assay provides a direct measurement of antioxidant capacity against hydrophilic chain breaking peroxyl radicals. This procedure is performed as described by Ou et al (2001) with some modifications (Girard-Lalancette et al 2009).

Materials and Methods

Fluoroskan plate reader equipped with 488nm excitation and a 515nm emission filters,

PC connected to plate reader (Username: datalog, Password: fluoroskan)

96-well plates (black and white isoplates- white wells, black matrix) Wallac, Perkin Elmer.

Pipettes with range 25-1000µl

Multichannel pipette with range of 25-150µl

Plasma samples must be obtained from Heparin tubes and diluted 1:100 in buffer before analysis. Can also do analysis in protein-free fraction using 0.5M perchloric

acid (PCA; in buffer). Add PCA and plasma in eppendorf (1:1, v/v) centrifuge at 13000g for 10 minutes at 4C, and treat as whole plasma.

Reagents **N.B.** PREPARE ALL REAGENTS AND SAMPLES IN PHOSPHATE BUFFER (pH 7.4, 75mM)

Perchloric Acid

- 2.16ml PCA made up to 50ml in buffer.

Fluorescein MW: 376.28

- Prepare a 40mM stock solution – weigh out 0.1505g fluorescein in 10ml buffer
    - This can last several months so long as stored at 5˚C covered in foil.
- Immediately prior to use, dilute stock solution to 400nM with buffer
    - Dilute stock solution 1:100 (0.1ml stock + 9.9ml buffer) to give 400µM solution,
    - Dilute this again 1:100 to give a 4µM solution,
    - Dilute this solution 1:10 (2ml of above + 18ml buffer) to give a 400nM solution.
- Protect from light by covering with foil and store at 5˚C.

AAPH (375mM) MW: 271.20

- Weigh 1.017g AAPH and dissolve in 10ml buffer
- Prepare fresh daily, store at 5˚C and discard within 8 hours.

Trolox MW: 250.29

- Weigh 0.0250g Trolox in 10ml buffer (10mM) and vortex until dissolved.
- Aliquot and store at -20˚C for 3 months. Defrost at room temperature.
- On the day, dilute this solution 1:100 to give 100µM (9.9ml buffer +0.1ml Trolox)
- Prepare serial dilutions for a calibration curve: 100, 50, 25, 12.5, 6.25µM.

Procedure

- Login to PC and enter password
- Switch on plate reader and allow to warm up.
- Open Fluoroskan Ascent icon
- Open a session (must be a HI.SEF file)
- Highlight the area of wells to be analysed and define wells.
- Add 25µl buffer to the BLANK wells, and 25µl sample/standard to each well + 150µl fluorescein (400nM) and place in fluoroskan.
- Press start. Ignore window that pops up, this will disappear after 15mins.

- Incubate sample in Fluoroskan at 37˚C for 15mins. Plate will automatically come out after this time.
- Add 25µl AAPH and press continue.
- Run time of assay is ~35min.
- Save data as an excel file.
- The final results are calculated using the net AUC of the sample concentrations. ORAC values are expressed in micromoles of Trolox equivalents (TE) per gram (µmol TE/g).

Thiobarbituric Acid Reactive Substances (TBARS) Assay

Cayman's TBARS Assay Kit

Sample Preparation –Plasma

Typical human plasma has a lipid peroxide level (expressed in terms of MDA) of 1.86-3.94 µmol.

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1000 x g for 10 minutes at 4ºC.
3. Plasma does not need to be diluted before assaying.

General Information

- All reagents except samples must be equilibrated to room temperature before beginning the assay. The SDS Solution will take at least one hour to equilibrate to room temperature if stored at 2-8ºC. Briefly heating the SDS Solution at 37ºC will re-dissolve the precipitated SDS. The SDS Solution can then be stored at room temperature.
- The final volume of the assay is 150 µL is all wells.
- The assay is performed at room temperature.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples and standards be assayed at least in duplicate.
- It is recommended that the samples and standards be kept at 4ºC after preparation to increase sensitivity and reproducibility.
- Monitor the absorbance at 530-540 nm or read fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 550 nm.

Reagents

- Thiobarbituric Acid (TBA)
- TBA Acetic Acid
- TBA Sodium Hydroxide
- TBA Malondialdehyde Standard
- TBA SDS Solution
- Colour Reagent:
    - Weigh 530 mg of TBA and add to ≥150 ml beaker containing 50 ml of diluted TBA Acetic Acid Solution. Add 50 mL of diluted TBA Sodium Hydroxide and mix until the TBA is completely dissolved. The solution is stable for 24h.

Calorimetric Standard Preparation

Dilute 250 µL of the MDA Standard (TBA Malondialdehyde) with 750 µL of water to obtain a stock solution of 125 µmol. Take eight clean glass test tubes and label them A-H. Add the amount of 125 µmol. Add the amount of 125 µmol stock solution and water to each tube as described in table 1.

Table 1. MDA calorimetric standards

Tube	MDA µmol	Water (µL)	MDA Concentration (µmol)
A	0	1000	0
B	5	995	0.625
C	10	990	1.25
D	20	980	2.5
E	40	960	5
F	80	920	10
G	200	800	25
H	400	600	50

Fluorometric Standard Preparation

Dilute 25 µL of the MDA Standard (TBA Malondialdehyde) with 975 µL of water to obtain a stock of 12.5 µmol. Take eight clean glass test tubes and label them A-H. Add the amount of 12.5 µmol MDA stock solution and water to each tube as described in table 2.

Table 2. MDA fluorometric standards

Tube	MDA µmol	Water (µL)	MDA Concentration (µmol)
A	0	1000	0
B	5	995	0.0625
C	10	990	0.125
D	20	980	0.25
E	40	960	0.5
F	80	920	1
G	200	800	2.5
H	400	600	5

Performing the Assay

1. Label vial caps with standard number or sample identification number.
2. Add 100 µL (1L was used here) of sample or standard to appropriately labelled 5 mL vial.
3. Add 100 µL (1 L was used here) of SDS Solution to vial and swirl to mix.
4. Add 4mL of Colour Reagent forcefully down side of each vial.

5. Cap vials (plastic caps tubes used -10 mL) and place them in foam or some other holder to keep the tubes upright during boiling.
6. Samples were boiled at 85-95ºC temperature in a water bath for 1 hour
7. After 1 hour immediately remove the vials and place in ice bath to stop reaction. Incubate on ice for 10 minutes
8. After 10 minutes, centrifuge the vials for 10 minutes at 1600 x g at 4ºC. Vials may appear clear or cloudy. Cloudiness will clear upon warming to room temperature –these were placed in Eppendorf tubes to make sure they cleared.
9. Vials are stable at room temperature for 30 minutes
10. Load 150µL (1 L here) in duplicate from each vial to either the clear plate (calorimetric version) or to the black plate (fluorometric version)
11. Read the absorbance at 530-540 nm or read fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 550 nm. It was read in cuvettes at 532 nm.

Calorimetric Calculations

1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of the standard A (0 µmol) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Plot the corrected absorbance values (from step 2 above) of each standard as a function of MDA concentration.
   MDA (µmol) = [(Corrected absorbance)-(y-intercept)/Slope]
4. Calculate the values of MDA for each sample from the standard curve.

Fluorometric Calculations

1. Calculate the average fluorescence of each standard and sample.
2. Subtract the fluorescence value of the standard A (0 µmol) from itself and all other values (both standards and samples). This is the corrected fluorescence.
3. Plot the corrected fluorescence values (from step 2 above) of each standard as a function of MDA concentration.
   MDA (µmol) = [(Corrected fluorescence)-(y-intercept)/Slope]
4. Calculate the values of MDA for each sample from the standard curve.

Performance characteristics

Precision:

When a series of ten human plasma were assayed on the same day, the intra-assay coefficient of variation was 5.5% and 7.6% respectively.

When a series of eight human plasma were assayed on seven different days under the same experimental conditions, the inter-assay coefficient of variation was 5.9% and 5.1% respectively.

Assay Range:

Under the standardised conditions of the assay described above, the dynamic range of the kit is 0-50 µmol (Calorimetric) or 0-5 µmol (Fluorometric) (µmol = µmol/L = nmol/mL) MDA equivalents.